920
884/A

JOURNAL OF ECONOMIC ENTOMOLOGY

ENTOMOLOGICAL SOCIETY OF AMERICA

Vol. 51 February, 1958 No. 1

The Conversion of Aldrin and Heptachlor to Their Epoxides in Soil[1]

NORMAN GANNON and J. H. BIGGER[2]

ABSTRACT

The insecticides aldrin and heptachlor have been found to epoxidize to dieldrin and heptachlor epoxide, respectively, in soils treated for control of soil-inhabiting insects. Such conversions may be assumed to be advantageous since the compounds produced are not only toxic but also are more residual than are the parent materials. This may partially explain the prolonged insecticidal efficiency obtained in soils treated with aldrin and heptachlor.

The conversion of heptachlor to its epoxide was first proved to occur in the animal body by Davidow & Radomski (1953). Similarly Bann *et al.* (1956) presented evidence showing the conversion of aldrin to dieldrin in animals. Recent evidence has proved that both materials convert to their epoxides on plants as well (Gannon & Decker, In press). Furthermore, Glasser (1956) found dieldrin residues on carrots grown in aldrin-treated beds.

Because of these findings, such a conversion was not considered unlikely in soils which had been treated with aldrin or heptachlor for the control of soil-inhabiting insects. Conversion in soil could partially explain the prolonged insecticidal efficiency of the treatments, since both epoxides, in addition to being highly toxic to insects, are more residual than are either of the relatively short-lived parent materials.

METHODS.—Field-sized treatments of aldrin or heptachlor were applied to soil and disked in to study the length of insecticidal efficiency of such treatments. Treated fields were sampled, their samples composited, and a representative sub-sample was extracted with either solutions of 5% acetone or 25% benzene in hexane. The extracts were concentrated just to dryness and taken up in hexane alone. Both aldrin and heptachlor were separated from their epoxides in chromatographic columns (20 mm. I.D.) which contained activated florisil. After adsorbing the sample onto the column, heptachlor or aldrin was eluted with 200 ml. of hexane. Their respective epoxides were eluted from the columns into a second flask with an additional 600 ml. of hexane. Heptachlor and heptachlor epoxide fractions were then analyzed according to the method of Davidow & Radomski (1953). Aldrin and dieldrin fractions were cleaned up further and analyzed according to O'Donnell *et al.* (1954, 1955). Colorimetric analyses were sensitive to below 0.01 p.p.m.

Samples similarly split and cleaned up were subjected to bioassay on houseflies (*Musca domestica* L.) using a modification of the residue method of Sun & Sun (1952).

RESULTS AND DISCUSSION.—Evidence of the conversion of aldrin to dieldrin and heptachlor to its epoxide in soil as indicated by specific colorimetric and bioassay methods of analysis is presented in table 1. Figure 1 gives further evidence of the conversion of heptachlor to its epoxide in

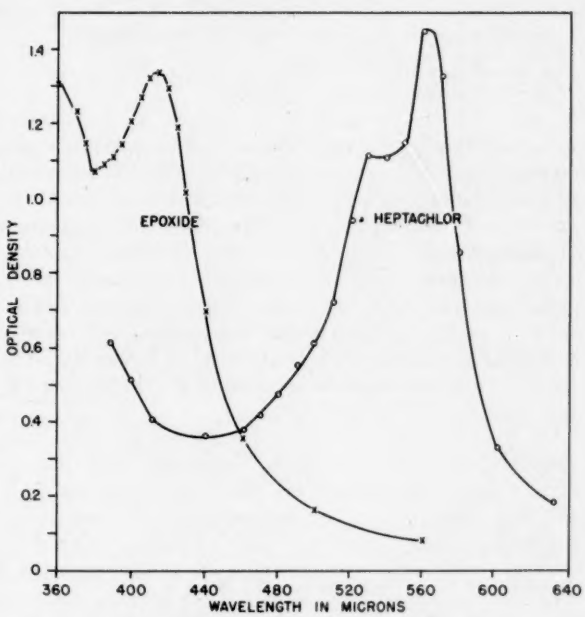


FIG. 1.—Absorption spectra of products obtained when heptachlor and heptachlor epoxide were reacted with Davidow reagent.

that it shows spectrographs obtained as a result of reacting heptachlor and heptachlor epoxide obtained from heptachlor-treated soil with Davidow reagent. These spectrographs show the same configuration as do those obtained when re-crystallized heptachlor and epoxide are reacted similarly.

From the data shown it is apparent that aldrin converts to dieldrin in soil somewhat more rapidly than does heptachlor to its epoxide. It is further evident, particularly with aldrin, that most of the prolonged toxicity to

[1] A contribution from North Central Regional Project NC-19. Accepted for publication October 18, 1957.

[2] Associate Entomologist and Entomologist, respectively, Illinois Natural History Survey, Urbana.

Table 1.—Parts per million aldrin, dieldrin, heptachlor, and heptachlor epoxide measured in soils treated with aldrin and heptachlor for soil-insect control.[a]

Treatment	Pounds per Acre	Years Applied[b]	Year Sampled[b]	Parts per Million				
				Aldrin	Heptachlor	Dieldrin	Heptachlor epoxide	Bioassay dieldrin or heptachlor epoxide[c]
Aldrin	1.5	1955	1956	0.01		0.10		
	2.0	1956	1956	.15		.37		0.31
	1.5, 2.0	1955, 1956	1956	.09		.45		.40
	1.5	1954	1956	.01		.08		
	1.5	1954, 1955	1956	.01		.11		
	1.5	1954, 1955, 1956	1956	.08		.45		.33
	1.0	1955	1956	.01		.05		
	1.0	1955, 1956	1956	.07		.27		.21
	1.5	1955	1956	.01		.03		
	10.0	1956	1956	1.62		.36		.40
Heptachlor	1.5	1954, 1955	1956		0.03		0.05	
	1.5	1954, 1955, 1956	1956		.48		.17	.19
	1.5	1954, 1955, 1956	1957		.036[d]		.022[d]	
	1.5	1954, 1955, 1956	1957		.026[d]		.025[d]	
	2.6	1957	1957		.074[d]		.014[d]	
	15.2	1956	1957		.422[d]		.163[d]	.20

[a] Sensitivity was 0.01 p.p.m. except where otherwise designated.
[b] Treated in spring, sampled in fall.
[c] Sensitivity was 0.05 p.p.m.
[d] Sensitivity was 0.02 p.p.m.

soil-inhabiting insects must be due to the presence of the dieldrin produced, since in all plots, with the exception of the 10-pound per acre treatment of aldrin in 1956, the amounts of dieldrin considerably exceeded the amounts of aldrin present. The quantities of heptachlor remaining after treatment generally exceeded the quantities of epoxide measured, but since the epoxide is probably more toxic to insects in general than heptachlor itself, as was definitely indicated in bioassay tests on house flies, the epoxide produced must be of considerable importance in controlling soil-inhabiting insects.

Such conversions of these insecticides in soils may, therefore, be assumed to be advantageous, since insecticidally active compounds are produced which not only are toxic but also are more residual than the parent materials.

References Cited

Bann, J. M., T. J. De Cino, N. W. Earle, and Y. P. Sun. 1956. The fate of aldrin and dieldrin in the animal body. Jour. Agric. and Food Chem. **4**(11): 937–41.

Davidow, Bernard, and J. L. Radomski. 1953. Isolation of an epoxide metabolite from fat tissues of dogs fed heptachlor. Jour. Pharmacol. and Exptl. Therapeutics. **107**(3): 259–65.

Gannon, Norman, and G. C. Decker. The conversion of heptachlor to its epoxide on plants. Jour. Econ. Ent. **51**(1): 3–7.

Gannon, Norman, and G. C. Decker. The conversion of aldrin to dieldrin on plants. Jour. Econ. Ent. **51**(1): 8–11.

Glasser, R. F. 1955. Evidence of the presence of a toxic metabolite of aldrin in carrot plant material. Thesis for Ph.D. Degree. Cornell University.

O'Donnell, A. E., M. M. Neal, F. T. Weiss, J. M. Bann, T. J. De Cino and S. C. Law. 1954. Chemical determination of aldrin in crop materials. Jour. Agric. and Food Chem. **2**(11): 573–80.

O'Donnell, A. E., H. W. Johnson, Jr. and F. T. Weiss. 1955. Chemical determination of dieldrin in crop materials. Jour. Agric. and Food Chem. **3**(9): 757–62.

Sun, Y. P., and J. Y. T. Sun. 1952. Microbioassay of insecticides, with special reference to aldrin and dieldrin. Jour. Econ. Ent. **45**(1): 26–36.

BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

Members should read this publication carefully!
It contains announcements, notices, etc.,
not sent to the membership in any other way.

The Conversion of Heptachlor to Its Epoxide on Plants[1]

NORMAN GANNON[2] AND G. C. DECKER[3]

ABSTRACT

The conversion of heptachlor to heptachlor epoxide in substantial quantities occurs on certain plants and may be common on all plants. A 1-pound per acre treatment of heptachlor on alfalfa yielded residues of heptachlor which persisted above 0.1 p.p.m. for 13 days while the epoxide produced did not reach this level until 25 days after treatment. This is of importance not only because the epoxide is the more persistent of the two but also because it has a higher insect and mammalian toxicity than the parent material, heptachlor.

The conversion has been proved by specific colorimetric, total organic chloride, bioassay, paper chromatographic, and infrared spectroscopic methods.

In pursuing a study on the rate of insecticide residue disappearance on growing crops over a period of years, the authors consistently noted a highly significant disparity between results obtained by total chlorine and specific methods of analysis employed for the evaluation of residues of heptachlor and aldrin. This paper and the paper immediately following it present findings developed to date in an attempt to account for the observed disparities.

Davidow & Radomski (1953) found that, when fed to dogs, heptachlor metabolizes to heptachlor epoxide which is stored in animal fat. Further evidence supporting and broadening these original findings was published by Davidow *et al.* (1953). Of all the other work dealing with the fate of insecticides in the animal body, the most similar involves the conversion of aldrin in animals to an epoxide which was proved to be dieldrin by Bann *et al.* (1956). It has also been shown that both heptachlor and aldrin are converted to their epoxides in soil (Gannon & Bigger 1958). Although there is no published evidence that a similar conversion takes place on plants, the fact that it does occur in animals and in soils suggested such a possibility should be explored.

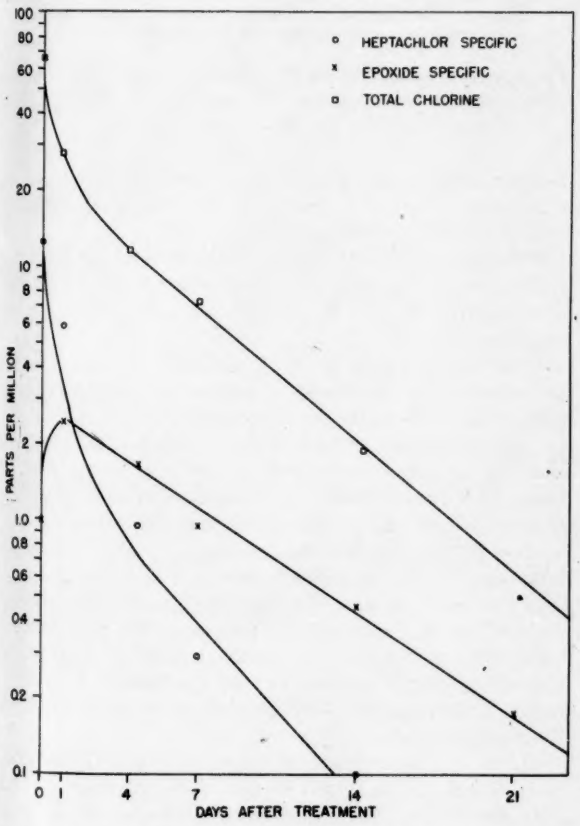


FIG. 1.—Persistence curves of heptachlor and heptachlor epoxide resulting from spraying alfalfa with 1 pound of heptachlor per acre.

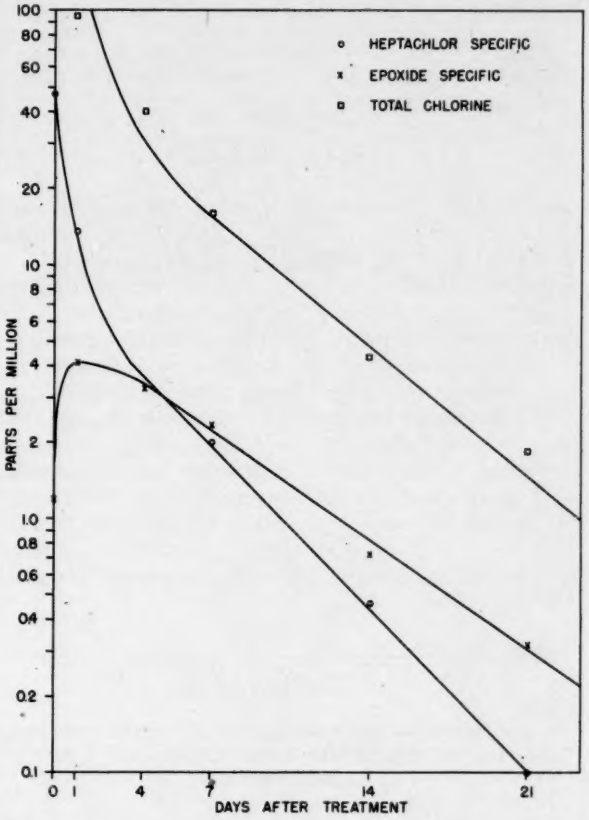


FIG. 2.—Persistence curves of heptachlor and heptachlor epoxide resulting from spraying alfalfa with 5 pounds of heptachlor per acre.

METHODS.—*Spray application.*—Sprays of heptachlor emulsion, formulated from recrystallized heptachlor, were applied to alfalfa at rates of 1 and 5 pounds per acre. These dosages, while in excess of amounts commonly used in insect control, were used to provide ample experimental material with which to work. Samples for analysis

[1] A contribution from North Central Regional Project NC-33. Accepted for publication November 26, 1957.

[2] Associate Entomologist, Illinois Natural History Survey, Urbana.

[3] Entomologist and Head, Section of Economic Entomology, Illinois Natural History Survey, Urbana.

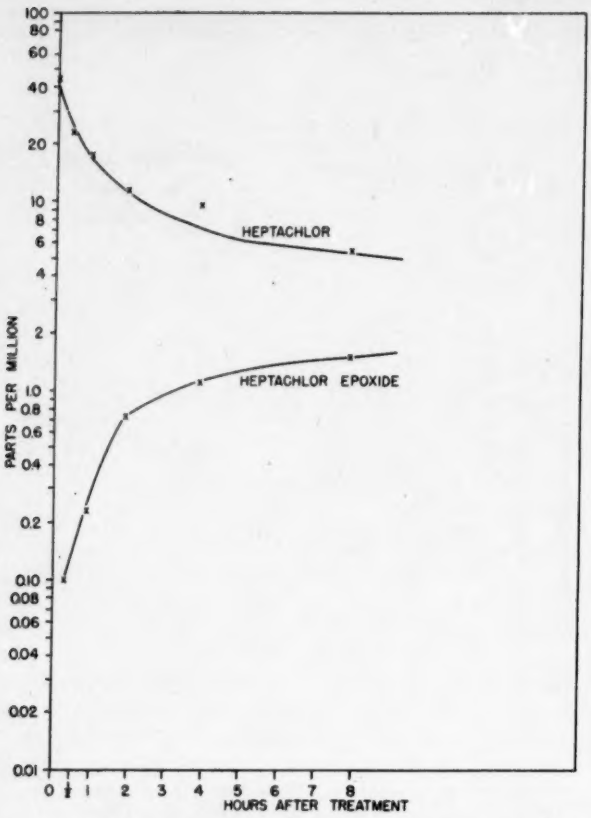


FIG. 3.—Heptachlor and heptachlor epoxide residue levels found during the first 8 hours after spraying alfalfa with 1 pound of heptachlor per acre.

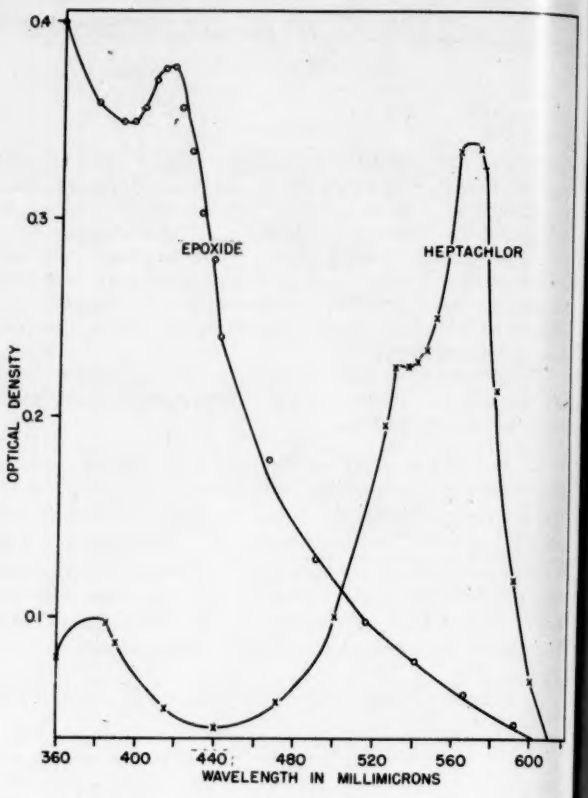


FIG. 4.—Absorption spectra of products obtained when heptachlor and heptachlor epoxide were reacted with Davidow reagent.

were taken at 0, 1, 4, 7, 14, and 21 days after spraying. The same formulation was applied later to corn and soybeans. A dosage of heptachlor in excess of 25 pounds per acre was also applied to soybeans so that sufficient material with a minimum of plant contaminants could be extracted for qualitative infrared analysis.

Extraction and clean-up.—Samples were extracted in either colorimetric grade pentane[4] or hexane[5] depending upon the method of analysis contemplated.

Cleanup of pentane extracts to be analyzed by the Polen-Silverman (1952) method was accomplished by first cooling the samples, then chromatographing them on columns of activated 100/200 mesh florisil (heated at 130° C. for 24 hours). The columns used were 20 mm. inner diameter×150 mm. long. Heptachlor was eluted from the column with 100 ml. of colorimetric grade pentane.

Hexane extracts to be analyzed by the method of Davidow & Radomski (1953) and by bioassay were also chromatographed on activated florisil columns. By the use of columns 20 mm. inner diameter, it was determined that heptachlor could be separated from its epoxide by eluting the heptachlor with 150 ml. of hexane and the epoxide (collected in a second flask) with an additional 800 ml. of hexane or 200 ml. of a solution of 25% benzene or 5% acetone in hexane. Generally, the latter two solvent combinations were not used in eluting the epoxide fraction from crop extracts since they were inclined to elute some of the crop residue itself. This was especially true of the 5% acetone solution.

Columns of diameters larger than 20 mm. held up the elution of the epoxide. Likewise, attempts to elute the epoxide from 20 mm. florisil columns with pentane were unsuccessful.

Colorimetric analysis.—The method of Davidow is based upon the treatment of heptachlor with a reagent consisting of 1 part diethanolamine and 2 parts 2N methanolic potassium hydroxide to form a violet product showing an absorbance peak at 560 mu. Treatment of heptachlor epoxide with this reagent results in a yellow product which has a peak at 415 mu. Quantitative estimation of this product can be made by the "base line technique" of Wright (1941) whereby absorbance readings are taken at 390, 415, and 440 mu. By subtracting the average of the densities obtained at 390 and 440 mu from that at 415 mu, the concentration of heptachlor epoxide originally present can be calculated. All colorimetric determinations were made on a Beckman Model B Spectrophotometer.

Dilute concentrations of heptachlor and heptachlor epoxide can be analyzed in the same solution by the Davidow-Radomski method since their adsorption peaks are widely separated. However, at higher concentrations

[4] Phillips Petroleum Company, Bartlesville, Oklahoma.
[5] Skellysolve "B," Skelly Oil Company, Chicago, Illinois.

Table 1.—Comparison of residue levels (parts per million) of the heptachlor present and the epoxide produced on alfalfa as determined by various methods of analysis.

Heptachlor Lb./Acre	Days After Treatment	Polen-Silverman Method	Davidow-Radomski Method		Bioassay		Total Chlorine Method
		Heptachlor	Heptachlor	Heptachlor Epoxide	Heptachlor	Heptachlor Epoxide	
1	0	—	12.13	0.99	—	—	67.1
	1	—	5.82	2.42	—	—	28.2
	4	—	0.95	1.65	—	—	11.5
	7	—	0.29	0.94	—	—	7.2
	14	—	0.1	0.45	—	—	1.9
	21	—	—	0.17	—	—	—
5	0	—	46.38	1.19	46.00	1.16	361.7
	1	11.27	13.5	4.11	—	4.52	94.8
	4	2.50	3.25	3.28	5.70	3.24	39.2
	7	1.61	2.06	2.35	2.45	1.80	15.7
	14	0.45	0.46	0.73	0.47	0.95	4.4
	21	0.14	0.1	0.32	0.26	0.57	1.8

Table 2.—Comparison of R_f values[a] obtained by paper chromatography from extracts of heptachlor-treated alfalfa.

Compound	Source	R_f	Color of Spots
Heptachlor	Reference grade	0.49	Violet
Heptachlor	Alfalfa	.48	Violet
Heptachlor epoxide	Reference grade	.57	Yellow
Heptachlor epoxide	Alfalfa	.59	Yellow

[a] $R_f = \frac{\text{distance covered by spot front}}{\text{distance covered by mobile solvent front}}$.

the violet of the heptachlor tends to overshadow the yellow of the epoxide. Consequently, the crop samples were always separated on florisil prior to analysis.

The Polen-Silverman method, used to substantiate heptachlor persistence curves on alfalfa, involves the treatment of heptachlor with a reagent consisting of ethanolamine in butyl cellosolve (Polen & Silverman 1952).

Other methods used to substantiate the presence of heptachlor epoxide in crop extracts included total organic chloride, bioassay, paper chromatography, and infrared analysis.

Total organic chloride analysis.—This method involves the liberation of chlorine by sodium reduction followed by the familiar Volhard titration for chlorine.

Bioassay.—Samples to be subjected to bioassay were first separated on a florisil column as described previously, heptachlor being eluted first and the epoxide second. Aliquots of epoxide fractions were treated with Davidow reagent prior to bioassay to insure that no heptachlor had trailed over into these fractions. Since it was established that all the heptachlor was eluted in the first fraction, any toxicity arising from exposing susceptible house flies to the remainder of the second fraction had to be due to a material other than heptachlor. Two to five

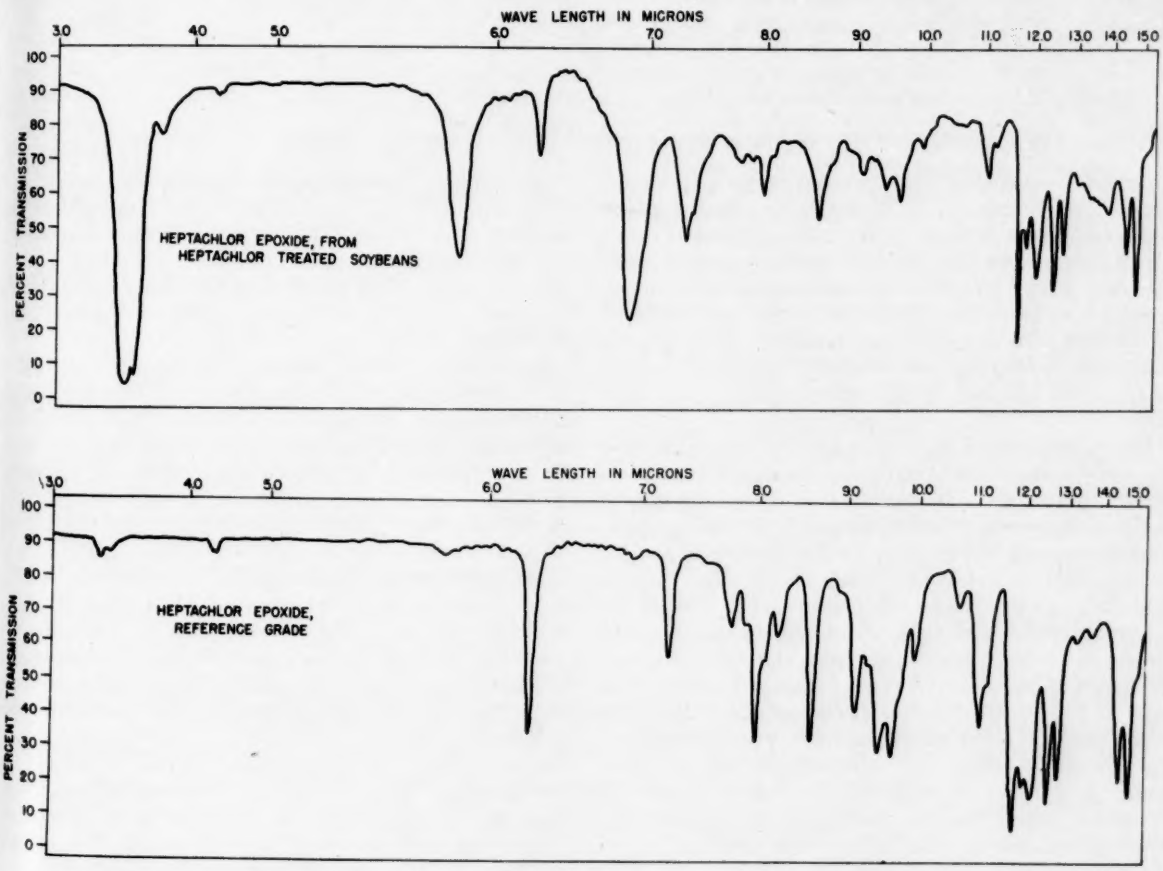
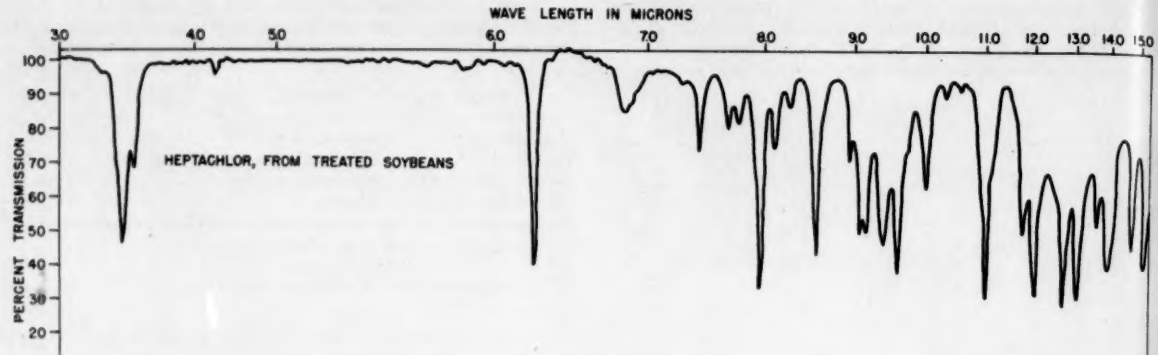


FIG. 5.—Infrared curves comparing heptachlor epoxide extracted from soybeans with reference grade heptachlor epoxide.



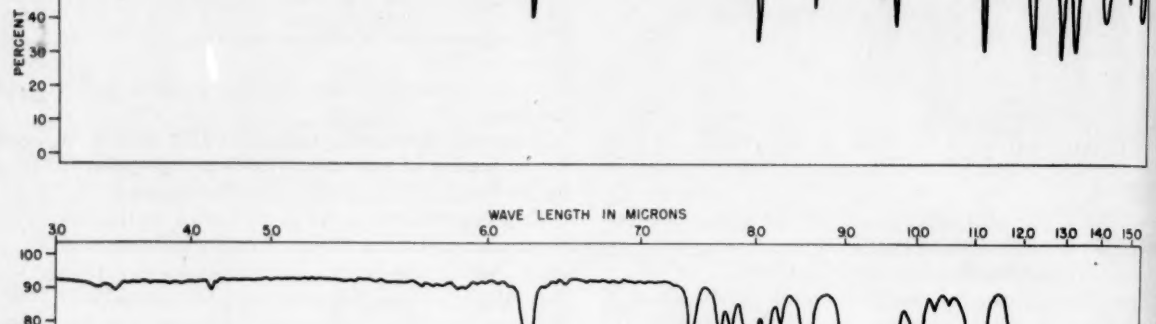


FIG. 6.—Infrared curves comparing heptachlor extracted from soybeans with reference grade heptachlor.

replicates consisting of 100 Chemical Specialties Manufacturers Association (CSMA) flies were exposed to the cleanedup alfalfa extracts in crystallizing dishes as patterned closely after the "residue" method of Sun (1952). Generally after 2 to 3 hours of exposure, readable, differential results could be taken. Quantitative estimation of the amount of toxicant present was made by comparing mortalities yielded by unknowns with logarithmic probability curves obtained from standards run at the same time.

Paper chromatographic separation of heptachlor from heptachlor epoxide.—R_f values were measured from spots developed from extracts of alfalfa treated with heptachlor and from standards of heptachlor and heptachlor epoxide. This was accomplished by using a modification of a method employed by Velsicol Chemical Corporation (unpublished). This method uses 1% soybean oil in ether as the stationary phase and 80% acetonitrile in water as the mobile phase. Spots are developed on the chromatograms by spraying with Davidow reagent and heating in an oven at 100° C. for 10 minutes. It was found that crop waxes present in the alfalfa extracts seriously hindered the movement of the spots of the paper. Consequently waxes were removed according to cleanup procedures described earlier, and the fractions were combined and concentrated before spotting the test solutions on the papers.

Infrared analysis.—Samples of high-dosage soybeans, cleaned up by methods previously described, were concentrated, dissolved in carbon tetrachloride or carbon disulfide and scanned on a Perkin-Elmer Recording Infrared Spectrophotometer Model 21 fitted with sodium chloride optics. They were compared with standard spectrograms scanned from reference grade heptachlor and heptachlor epoxide.

RESULTS AND DISCUSSION.—The presence of other products as a result of spraying recrystallized heptachlor on alfalfa was first suspected when analyses specific for heptachlor turned out to be considerably lower than those obtained by a total chlorine method of analysis. These differences could not be due to chlorides inherent in the crop itself since unsprayed check plots of alfalfa yielded no chlorine. Nor could they be attributed to other possible chlorine-containing constituents in the heptachlor emulsion concentrate applied since (1) composition analyses of formulation itself by both specific and total chlorine methods were in close agreement and (2) plots sprayed with an identical emulsifiable concentrate containing no heptachlor showed no measurable chlorine.

Consequently, differences in results obtained by the two methods would indicate the presence of a degradation product or products of heptachlor itself. The most logical approach appeared to be to check first for the presence of heptachlor epoxide in the crop extracts since heptachlor is converted to the epoxide in animals and

stores as such in animal fat. That such a conversion also takes place on alfalfa is evident from the data presented in table 1 which shows a comparison of quantitative data obtained by methods previously described. All residues are reported in parts per million on a green-weight basis. Figures 1 and 2 show persistence curves of heptachlor and heptachlor epoxide obtained from specific and total chemical analyses following the treatment of alfalfa with 1 and 5 lbs. of recrystallized heptachlor per acre.

Table 1 shows good agreement between the Polen-Silverman, Davidow-Radomski, and bioassay methods used for the quantitative determination of heptachlor. Chemical and bioassay methods for the determination of heptachlor epoxide also are in close agreement.

Of particular interest is the comparison of the heptachlor curves with those of the epoxide produced as a residue. As figure 1 shows (1 lb. heptachlor per acre), heptachlor persisted above 0.1 p.p.m. for 13 days while the epoxide produced did not reach a 0.1 p.p.m. level for approximately 25 days after treatment. It is also important to note that as early as 2 to 3 days after treatment the epoxide produced was of a greater magnitude in terms of parts per million than was the heptachlor remaining.

While these curves result from dosages greater than those normally used in insect control, the amounts of epoxide produced by lower dosages should be in proportion to those produced by high ones and the dissipation of the epoxide should exhibit the same slope as do the curves presented.

Figure 3 indicates the progress of conversion in its initial stages on a later alfalfa treatment. As heptachlor decreases during the first day after treatment, the epoxide increases, reaching its maximum concentration within 24 hours.

Paper chromatography further indicated the presence of heptachlor epoxide in alfalfa extracts. The close agreement of R_f values obtained from heptachlor and epoxide standard solutions with those obtained from heptachlor-treated alfalfa extracts is shown in table 2.

Spectrograms determined on a Beckman Model B Spectrophotometer offer further evidence of the conversion. Figure 4 shows the curves obtained when heptachlor and its epoxide extracted from alfalfa were treated with Davidow reagent. The peaks obtained (at 415 mu for heptachlor epoxide and at 560 for heptachlor) are identical to those obtained on pure heptachlor and heptachlor epoxide.

The analysis of cleanedup, high-dosage soybean extracts by infrared spectrography affords further proof that heptachlor is converted into its epoxide on plants. With the exception of three contaminant peaks, the spectrogram obtained from the epoxide fraction following a heptachlor treatment is identical to the one run on recrystallized heptachlor epoxide (see figure 5). Two of the three contaminant peaks are probably due to the hexane used in extraction and cleanup. These occur at 6.8 to 6.9 and at 3.3 to 3.5 microns. The other contaminant peak (at 5.7 to 5.9 microns) is probably an ester extracted from the plant which was not removed in the cleanup processes.

Figure 6 compares spectrograms of the heptachlor obtained from the crop with recrystallized heptachlor. Again the solvent influence is seen but to a much lesser extent. With that exception the spectrograms again are identical.

It appears the conversion of heptachlor to its epoxide may be common on plants in general since, in addition to the conversion on alfalfa, it has also been found to take place on soybeans and corn. It is further apparent, from the limited amount of work done in this regard, that two factors at least are necessary for conversion, i.e., the presence of plant material and light. The important constituents of either factor may be suspected but as yet are largely unproved.

REFERENCES CITED

Bann, J. M., T. J. DeCino, N. W. Earle, and Y. P. Sun. 1956. The fate of aldrin and dieldrin in the animal body. Jour. Agric. Food Chem. **4**(11): 937–41.

Davidow, Bernard, and J. L. Radomski. 1953. Isolation of an epoxide metabolite from fat tissues of dogs fed heptachlor. Jour. Pharmacol. and Exptl. Therap. **107**(3): 259–65.

Gannon, Norman, and J. H. Bigger. 1958. The conversion of aldrin and heptachlor to their epoxides in soil. Jour. Econ. Ent. **51**(1): 1–2.

Polen, P. B., and Paul Silverman. 1952. Detection and determination of 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene. Analyt. Chem. **24**: 733–5.

Radomski, J. L., and Bernard Davidow. 1953. The metabolite of heptachlor, its estimation, storage, and toxicity. Jour. Pharmacol. and Exptl. Therap. **107**(3): 266–72.

Sun, Y. P., and J. Y. T. Sun. 1952. Microbioassay of insecticides, with special reference to aldrin and dieldrin. Jour. Econ. Ent. **45**(1): 26–36.

Velsicol Chemical Corporation Project Report. The separation of heptachlor from range grass by partition paper chromatography. Unpublished.

Wright, Norman. 1941. Application of infrared spectroscopy to industrial research. Indust. and Engineer. Chem., Analyt. Ed. **13**(1): 1–8.

SUPPORT ALL OF YOUR SOCIETY'S PUBLICATIONS!

Keep up to date by purchasing the *Annual Review of Entomology, Entoma,* and the *Indexes to the Literature of American Economic Entomology.* Orders should be sent to:

Entomological Society of America
1530 P St., N. W.
Washington 5, D. C.

The Conversion of Aldrin to Dieldrin on Plants[1]

NORMAN GANNON[2] AND G. C. DECKER[3]

ABSTRACT

The conversion of aldrin to the more residual and equally toxic material, dieldrin, has been found to occur on alfalfa, soybeans, and corn and may take place on plants in general. A 1-pound per acre treatment of aldrin on alfalfa yielded aldrin residues which had dissipated to 0.1 p.p.m. in 8 days after spraying while the dieldrin produced took 25 days to reach the same level.

This conversion has been proved by specific colorimetric, total organic chloride, bioassay, paper chromatographic, and infrared spectroscopic methods.

Aldrin is known to be metabolized in the bodies of animals to an epoxide derivative, dieldrin, and to be stored as such in animal fat (Bann et al. 1956). Similarly the insecticide heptachlor also is converted to an epoxide and is stored in the same manner (Davidow *et al.* 1953). Glasser (1955) has shown further that carrots taken from aldrin-treated beds bore dieldrin residues. It has further been established that both aldrin and heptachlor epoxidize to toxic metabolites in soil (Gannon & Bigger 1958). That heptachlor is converted into an epoxide not only in animals and in soils but also on plants has been shown by the authors in the paper immediately preceding this one.

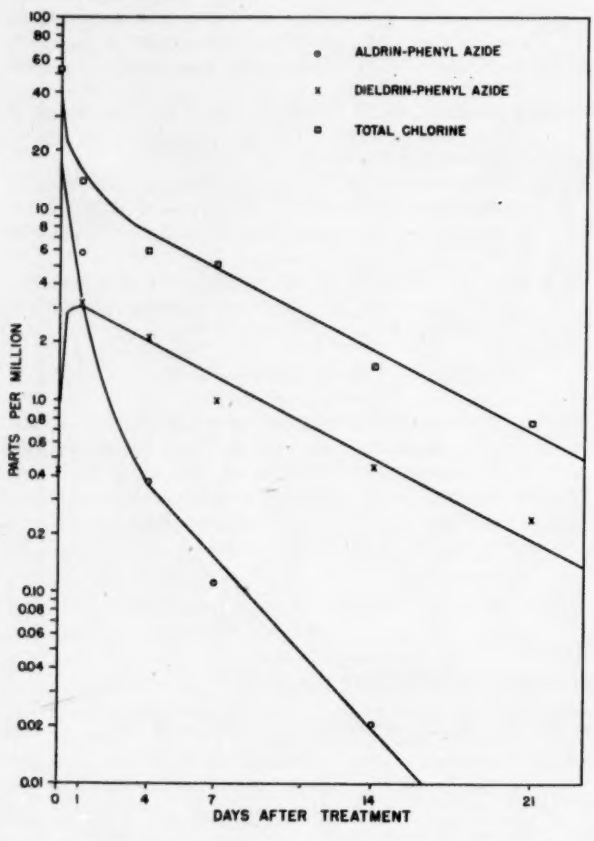


FIG. 1.—Persistence curves of aldrin and dieldrin resulting from spraying alfalfa with 1 pound of aldrin per acre.

Evidence is given in this paper which indicates the expoxidation of aldrin on plants to form dieldrin.

METHODS.—*Spray Application.*—Prior to treatment, the aldrin emulsifiable concentrate was checked to insure that it contained no dieldrin. This was done by separating a sample on a florisil column as described under the section "Extraction and cleanup." Showing no dieldrin to be present, sprays were applied to alfalfa at rates of approximately 1 and 5 pounds per acre. Samples were taken at 0, 1, 4, 7, 14, and 21 days after treatment. At a later date, to verify a possible conversion on other crops, aldrin was applied to corn and soybeans.

Table 1.—Comparison of residue levels of aldrin present and dieldrin produced on alfalfa as determined by various methods of analysis.

ALDRIN (LB./ ACRE)	DAYS AFTER TREATMENT	PHENYL AZIDE METHOD		BIOASSAY	TOTAL CHLORIDE METHOD
		ALDRIN	DIELDRIN	DIELDRIN	
		Parts per Million			
1	0	34.4	0.42	—	51.5
	1	5.9	3.24	—	14.1
	4	0.37	2.11	—	6.0
	7	0.11	0.98	—	5.1
	14	0.02	0.44	—	1.5
	21	—	0.24	—	0.8
5	0	155.3	3.10	4.2	231.1
	1	20.5	7.80	8.8	54.2
	4	1.99	4.06	—	24.0
	7	0.48	3.10	3.2	15.4
	14	0.09	1.81	1.4	5.8
	21	—	0.71	0.6	4.4

Table 2.—Comparison of R_f values[a] obtained by paper chromatography from extracts of aldrin-treated alfalfa.

COMPOUND	SOURCE	R_f VALUES
Aldrin	Recrystallized	0.41
Aldrin	Alfalfa	.40
Dieldrin	Recrystallized	.62
Dieldrin	Alfalfa	.63

[a] $R_f = \frac{\text{Distance to center of spot}}{\text{Distance to mobile solvent front}}$.

Extraction and Cleanup.—All aldrin-treated crops were extracted with hexane.[4] Extracts were dried over anhydrous sodium sulfate before concentrating them for chromatography. Prior to chromatographing samples for aldrin analysis on attasol:celite (3:2) or samples for dieldrin analysis on activated magnesia:celite (2:1) as recommended by O'Donnell *et al.* (1954, 1955), aldrin

[1] A contribution from North Central Regional Project NC-33. Accepted for publication November 26, 1957.

[2] Associate Entomologist, Illinois Natural History Survey, Urbana.

[3] Entomologist and Head, Section of Economic Entomology, Illinois Natural History Survey, Urbana.

[4] Skellysolve "B," Skelly Oil Company, Chicago, Illinois.

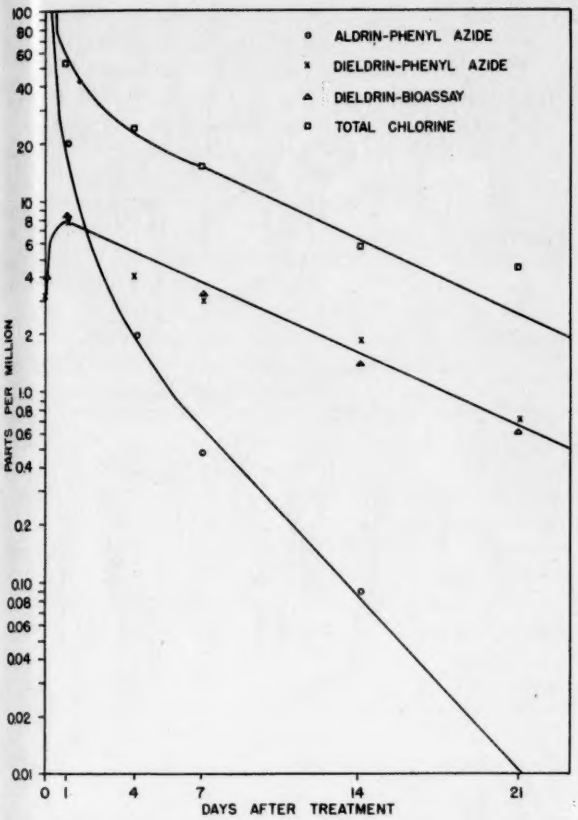


FIG. 2.—Persistence curves of aldrin and dieldrin resulting from spraying alfalfa with 5 pounds of aldrin per acre.

was separated from dieldrin by separating on a florisil column. This step was found necessary since a direct submission of an aldrin sample into a dieldrin analysis does not result in complete break-down of the aldrin present. Consequently a small percentage of it shows up as apparent dieldrin in the analysis. The separation was accomplished as follows: Using columns of 20 mm ID, 100/200 mesh activated florisil (heated at 130° C. for 24 hours) was packed to a height of 15 cm. All of the aldrin from a sample placed in the column was eluted with 150 ml. of hexane. Any dieldrin present was collected in a second flask by eluting further with 800 ml. of hexane.

Florisil chromatography eliminated most of the interfering plant materials. To insure thorough cleanup, however, aldrin fractions were chromatographed on attasol: celite, and dieldrin fractions on activated magnesia: celite.

Colorimetric analysis.—Samples were analyzed for both aldrin and dieldrin according to the phenyl azide method of O'Donnell *et al.* (1954, 1955). For aldrin this involves reacting the concentrated eluate from the chromatographic columns with phenyl azide to form an aldrin dihydrophenyl triazole, removal of the excess phenyl azide under reduced pressure, dissolving the residue in ethanol, acidifying with HCl, coupling the reaction product with 2,4-dinitroaniline, adding sulfuric acid and reading at 515 millimicrons. Dieldrin eluate is first treated with hydrobromic acid and acetic anhydride to break the epoxide ring and form a bromo-acetoxy derivative. Zinc dust is added and the mixture heated to reduce the bromo-acetoxy derivative to partially dechlorinated aldrin. From this point the analysis is identical to that described for aldrin.

Bioassay.—Aldrin and dieldrin in bioassay samples were separated on a florisil column as described previously. Aliquots of the dieldrin fractions thus obtained were analyzed chemically to insure that aldrin was absent. The remainder of the sample was used in bioassay patterned after the "residue" method of Sun (1952). Two to five replicates each consisting of 100 Chemical Specialties Manufacturers Association flies were exposed to the cleaned-up extracts evaporated to dryness in crystallizing dishes. Results were read within 2 to 3 hours after start of exposure and compared with standards run at the same time.

Paper chromatographic separation of aldrin from dieldrin.—Further substantiation of a possible conversion of aldrin to dieldrin was shown by paper chromatographs developed after the method of Mitchell (1953). Cleaned-up aldrin and dieldrin fractions were combined, concentrated, and spotted on 1-inch strips of Whatman No. 1 filter paper. The stationary phase consisted of a 1% solution of refined soybean oil in ethyl ether while the mobile phase consisted of a 60% by volume solution of pyridine in water. Chromatographs were developed with 0.05N

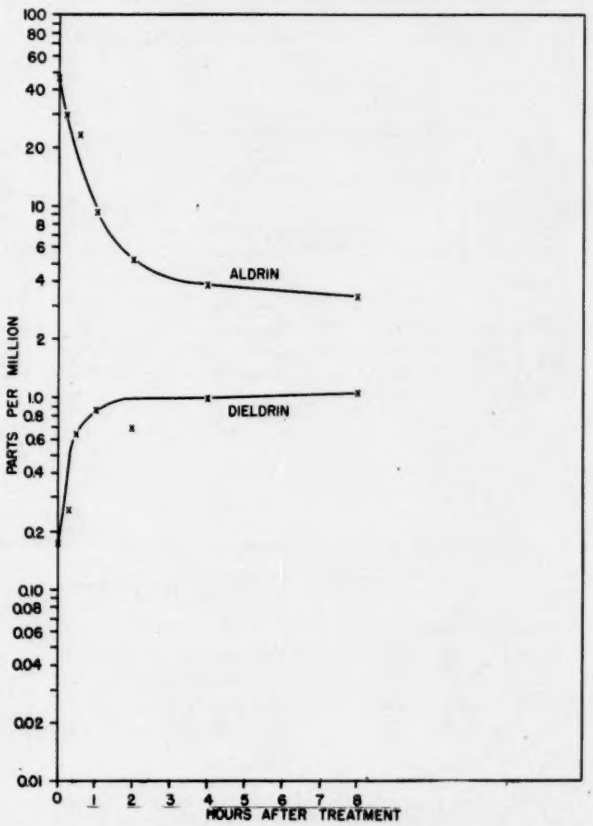


FIG. 3.—Aldrin and dieldrin residue levels found during the first 8 hours after spraying alfalfa with 1 pound of aldrin per acre.

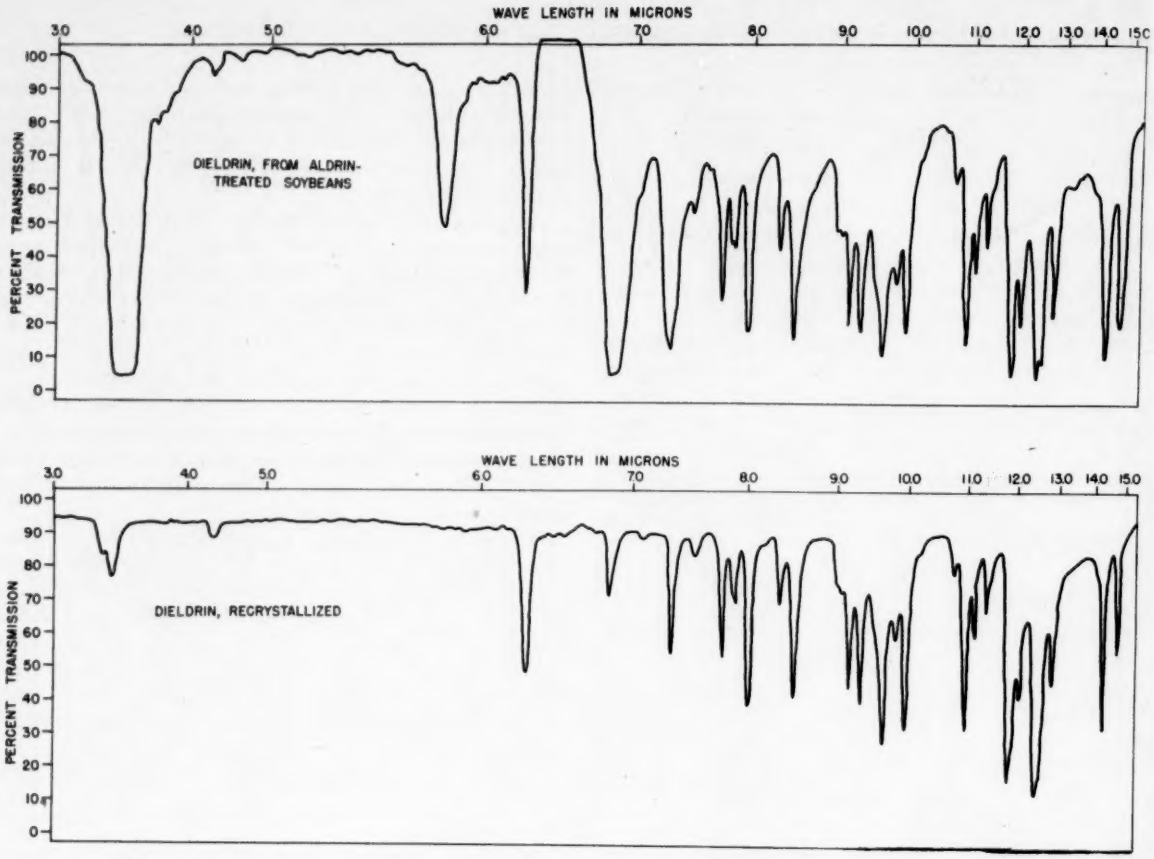

WAVE LENGTH IN MICRONS
3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
PERCENT TRANSMISSION
0 10 20 30 40 50 60 70 80 90 100
DIELDRIN, FROM ALDRIN-TREATED SOYBEANS
WAVE LENGTH IN MICRONS
3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
PERCENT TRANSMISSION
0 10 20 30 40 50 60 70 80 90 100
DIELDRIN, RECRYSTALLIZED

FIG. 4.—Infrared curves comparing dieldrin extracted from soybeans with recrystallized dieldrin.

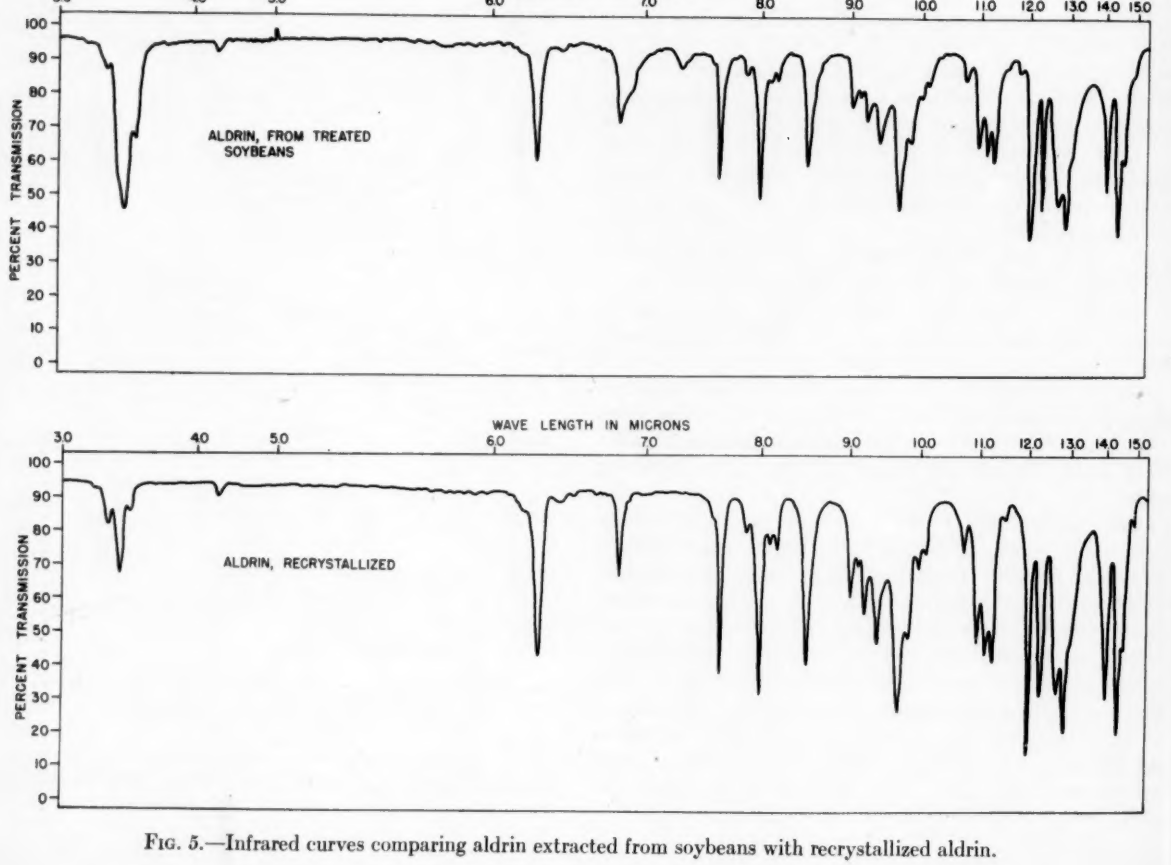

WAVE LENGTH IN MICRONS
3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
PERCENT TRANSMISSION
0 10 20 30 40 50 60 70 80 90 100
ALDRIN, FROM TREATED SOYBEANS
WAVE LENGTH IN MICRONS
3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
PERCENT TRANSMISSION
0 10 20 30 40 50 60 70 80 90 100
ALDRIN, RECRYSTALLIZED

FIG. 5.—Infrared curves comparing aldrin extracted from soybeans with recrystallized aldrin.

silver nitrate in ethanol, 37% formaldehyde, 2N potassium hydroxide, heat, and sunlight.

Infrared analysis.—Samples of soybean foliage, collected 1 day after treatment with aldrin at approximately 25 pounds per acre, were cleaned up as described previously, concentrated, dissolved in carbon tetrachloride or carbon disulfide and scanned on a Perkin-Elmer Recording Infrared Spectrophotometer Model 21 fitted with sodium chloride optics. Their spectrograms were compared with standard spectrograms obtained by scanning recrystallized aldrin and dieldrin.

RESULTS AND DISCUSSION.—Of the quantitative methods used in determining dieldrin residues on alfalfa as a result of treating with aldrin, the phenyl azide and bioassay methods yielded results which were in close agreement. (See table 1.) Figures 1 and 2 indicate the dissipation of aldrin on alfalfa and the production and dissipation of dieldrin. All residues are reported in parts per million on a green-weight basis. The 1-pound per acre treatment yielded 0.1 p.p.m. aldrin residue at 8 days after treatment while the dieldrin produced had not dissipated to that level for 25 days. While both dosages used were over-treatments, the amounts of dieldrin expected to be produced by lower dosages should be proportional and should exhibit the same slope as do the curves presented. In figure 2 the dieldrin values obtained by both phenyl azide and bioassay methods are so similar that one curve represents both.

Figure 3 shows the production of dieldrin and the dissipation of aldrin on alfalfa during the first 8 hours following a 1-pound per acre aldrin treatment as determined by the phenyl azide method.

Paper chromatography after the method of Mitchell (1953) supported existing evidence of a conversion in that two spots developed on papers spotted with cleaned-up crop extracts. If aldrin had been the only material present, only one spot would have developed. The distances which the aldrin and suspected dieldrin spots travelled up the papers were identical to distances travelled by recrystallized aldrin and dieldrin. (See table 2.)

Positive proof of the production of dieldrin on a plant as a result of treating with aldrin was afforded when high-dosage cleanedup soybean extracts were scanned in the infrared. With the exception of three contaminant peaks, the spectrograms are identical (figures 4 and 5). Peaks occurring at 6.8 to 6.9 and 3.3 to 3.5 microns probably result from the hexane used in extraction while the peak at 5.7 to 5.9 microns is probably a plant ester which came through the cleanup processes.

The conversion of aldrin to dieldrin has also been found to occur on corn. Since it is known to take place on corn, alfalfa, and soybeans it is not unreasonable to expect that conversion may occur on plants in general.

REFERENCES CITED

Bann, J. M., T. J. De Cino, N. W. Earle, and Y. P. Sun. 1956. The fate of aldrin and dieldrin in the animal body. Jour. Agric. Food Chem. **4**(11): 937–41.

Davidow, Bernard, and J. L. Radomski. 1953. Isolation of an epoxide metabolite from fat tissues of dogs fed heptachlor. Jour. Pharmacol. and Exptl. Therap. **107**(3): 259–65.

Gannon, Norman, and J. H. Bigger. The conversion of aldrin and heptachlor to their epoxides in soil. Jour. Econ. Ent. **51**(1): 1–2.

Gannon, Norman, and G. C. Decker. The conversion of heptachlor to its epoxide on plants. Jour. Econ. Ent. **51**(1): 3–7.

Glasser, F. 1955. Evidence of the presence of a toxic metabolite of aldrin in carrot plant material. Thesis for Ph.D. Degree. Cornell University.

Mitchell, L. C. 1953. Separation and identification of chlorinated organic pesticides by paper chromatography II. Aldrin and dieldrin. Jour. Assoc. Offic. Agric. Chemists **36**(2): 553–8.

Mitchell, L. C. 1953. Separation and identification of chlorinated organic pesticides by paper chromatography III. Aldrin, isodrin, dieldrin and endrin. Jour. Assoc. Offic. Agric. Chemists **36**(4): 1183–6.

O'Donnell, A. E., M. M. Neal, F. T. Weiss, J. M. Bann, T. J. De Cino, and S. C. Lau. 1954. Chemical determination of aldrin in crop materials. Jour. Agric. Food Chem. **2**(11): 573–80.

O'Donnell, A. E., H. W. Johnson, Jr., and F. T. Weiss. 1955. Chemical determination of dieldrin in crop materials. Jour. Agric. Food. Chem. **3**(9): 757–62.

Sun, Y. P., and J. Y. T. Sun. 1952. Microbioassay of insecticides, with special reference to aldrin and dieldrin. Jour. Econ. Ent. **45**(1): 26–36.

The Persistence of Heptachlor in Soils[1]

WILLIAM R. YOUNG[2] and W. A. RAWLINS[3]

The problems associated with the accumulation of insecticide residues in the soil, and the harmful effects of these residues upon the crops grown in these contaminated soils, have been the subject of renewed interest since the development of the synthetic organic insecticides. A proportion of the chemical directed at crop foliage in the usual manner of field application unavoidably sifts through the foliar cover to the soil surface. In other instances insecticides are applied directly to the soil for the control of subterranean pests. It is essential that we have information on the residual action in soils of the insecticides that are recommended for widespread use.

Each insecticide is specific in its residual properties. Other factors known to be involved in this problem are soil type and soil organic matter (Fleming 1950, Fleming & Maines 1953, 1954), and the depth to which a particular material has been incorporated in the soil (Kirk 1952, Fleming & Maines 1953, 1954).

[1] This paper constitutes part of a thesis presented by the senior author in partial fulfillment of the requirements for the Ph.D. degree at Cornell University, Ithaca, N. Y., supported in part by funds made available by Velsicol Corp., Chicago, Ill. Accepted for publication June 7, 1957.

[2] At present Assistant Entomologist with the Mexican Agricultural Program of the Rockefeller Foundation.

[3] Professor of Entomology, Cornell University.

The study reported here was undertaken to determine the persistence of heptachlor in the soil. Experiments were conducted to study how long this compound will persist when incorporated in the soil and when sprayed on the soil surface. Three mineral soils and an organic soil were included in the study. Other factors investigated included the effects of formulation, soil cultivation, and the exposure of treated soils surfaces to the sun upon the persistence of this insecticide. The possible vertical movement of heptachlor through the soil by action of water was also investigated.

At the onset of this study little information was available concerning the residual properties of heptachlor in the soil. Terriere & Ingalsbe (1953) determined the residue that persisted in a sandy loam soil 1 year after a treatment of 10 pounds per acre had been roto-tilled into field plots. A mosquito larvae bioassay of soil extracts indicated that 2.2 pounds of heptachlor still persisted and a total chloride chemical analysis detected 2.5 pounds per acre. Bigger (1955) found that a delay of 3 to 4 hours between the time heptachlor was applied to a field and mixed with the soil, reduced its effectiveness against root feeding insects by one-half, suggesting a rapid loss of this insecticide from the soil surface.

MATERIALS AND GENERAL METHODS.—All heptachlor soil treatments were made from emulsion and wettable powder formulations containing 25% of the active ingredient. Reference standard heptachlor was used in the preparation of standard solutions for the chemical analysis and bioassay of soil samples.[4]

Three mineral soils, Dunkirk sandy loam, Dunkirk silt loam and Dunkirk silty clay loam, all present in the vicinity of Ithaca, N. Y., and a muck (peat) soil from Oswego County, N. Y., were used. Since it has been suggested that organic matter and texture influence the residual action of insecticides, these properties for the soils involved were determined. Volume weights were also determined so that the residues detected could be converted from parts per million to pounds per acre and compared directly between soil types. The organic matter determinations were made by the Cornell soil testing laboratory and the mechanical analyses of the mineral soils were determined by the Bouyoucos (1936) hydrometer method. Volume weights were also determined through measurements of soil cores taken from the various soils in their natural field state. These soil properties are summarized in table 1.

All soil samples for chemical analysis were air dried and extracted in quart Mason jars with a solvent mixture of pentane, isopropanol and glacial acetic acid in the ratio 4:1:1 by volume. Samples of about 500 grams of mineral soil and 200 grams of muck were used for each extraction. The solvent was added to the samples at the ratio of 1 ml. per gram of mineral soil and 2 ml. per gram of muck. The samples with the solvent were agitated on a mechanical shaker for 30 minutes. They were then allowed to stand at room temperature for 24 hours, at which time the supernatant solvent was recovered and washed with distilled water to remove the alcohol and acid. A drying agent, anhydrous sodium sulfate, was added to the pentane extracts before storage in a cold room at 40° F. Aliquots of these extracts were later chromatographed in columns containing oven dried Florisil (60/100 mesh, Flordin Co., Warren, Pa.) to remove substances which interfered with the colorimetric determination specific for heptachlor (Polen & Silverman 1952, Ordas, *et al.* 1954).

The re-extraction of soil samples of each type indicated that recoveries of heptachlor were in excess of 95%. The recovery of heptachlor in the washing and chromatographic procedures was 95 to 100%. The estimated mean error of the colorimetric method was ±2.8% of the heptachlor present. Since values for the untreated soil samples fell within the range of reagent blanks (5 to 15 micrograms) and the residues in the treated samples were all above this range, no corrections were made for the check samples.

The residues in all samples were determined by the colorimetric chemical analysis. The analysis of soil extracts provides information on the quantity of a particular toxicant that is removed by an organic solvent used in the extraction procedure. It may not however supply an accurate estimate of the amount of the residue present that is available and toxic to living organisms in the soil, and it may not detect toxic degradation products. Therefore, the toxic residues in some of the samples of each soil type were determined with a bioassay.

In the bioassay employed the insecticide treated soil was mixed with applesauce, spread on paper toweling and exposed to adult fruit flies, *Drosophila melangoaster* Meig., 4 to 28 hours of age. The applesauce in the mixtures served as food for the flies. Pyrex test-tubes were used for holding vessels. The flies were reared as described by Olson (1954) and the bioassay conducted under controlled conditions of 70° F. and 60% relative humidity. The heptachlor present in each unknown sample was determined with 100 flies used in five replicates of 20 flies each. Mortality counts were taken after an exposure of 12, 24 and 48 hours. The criterion of death was complete immobility.

Standards were prepared by adding acetone solutions of reference standard heptachlor to untreated soil before the applesauce was added. With these standards an eye fitted dosage mortality curve was determined with each day's test. The amount of heptachlor present in the unknown samples was interpolated from these standard curves. This method is a modification of one in use in the Department of Entomology at Cornell University since 1951 for the assay of insecticide residues in crops.

INSECTICIDE INCORPORATED IN THE SOIL.—These studies involved treating soils of three types and measuring the amount persisting over a period of 21 months, where these soils were exposed to natural weathering.

During the summer of 1953 field plots were treated with heptachlor emulsion at the rate of 2 and 4 pounds per acre. These treatments were replicated twice on each of the three soil types: Dunkirk sandy loam; Dunkirk silty clay loam soils on the Experiment Station Farm and a muck soil near Oswego, N. Y.

The soil in these field plots at the three locations was sampled periodically for chemical analysis of the heptachlor present. The field sampling procedure used was adapted from that described by Koblitsky & Chisholm (1949). Approximately 50 cores, 2×6 inches, were taken

[4] All material supplied by Velsicol Corp., Chicago, Ill.

Table 1.—The physical characteristics of the soils included in the study of the persistence of heptachlor in soils.

Soil Type	Volume Weight (Gm./Cc.)	Mechanical Analysis			Organic Matter (Per Cent)
		Per Cent Sand (0.1–.05 mm.)	Per Cent Silt (0.05–.002 mm.)	Per Cent Clay (>.002 mm.)	
Dunkirk Sandy Loam	1.39	70.7	16.5	12.8	1.3
Dunkirk Silt Loam	1.05	26.4	50.0	23.6	1.0
Dunkirk Silty Clay Loam	1.40	16.4	48.0	35.6	3.9
Oswego County Muck	.40	—	—	—	90[a]

[a] The muck soil was estimated to be better than 90 per cent organic matter.

at random throughout the plot with a soil sampler of the type described by Johnson & Caskey (1952). These cores were placed in a galvanized bushel tub and thoroughly mixed. Then sufficient cores to fill a 1-gallon paper carton were taken from the tub with the same sampler. From the gallon container the number of 1-inch cores necessary to fill a 1-quart Mason jar were taken with a 1-inch soil sampling tube. These quart samples were held in a 40° F. cold room. Later they were air dried and then analyzed by the chemical method described above.

The variance associated with the sampling of field plots for insecticide residues is known to be great. In an attempt to reduce this sampling variance quantities of soil of the three types were collected from the field plot locations and treated with heptachlor at the laboratory. This soil was sifted through a ¼-inch mesh screen to remove the coarse matter present and was treated at the rates of 2 and 4 pounds per acre with the emulsion formulation on a weight-volume basis. In the process of treating, the emulsion containing the insecticide was layered into the soil in a galvanized bushel container. The soil was then twice run through a soil shredder (Lindig Model B) to make the treated soil as homogenous as possible with respect to heptachlor content. This treated soil was then placed in a galvanized metal cylinder 15 inches in diameter and 18 inches deep, that had been painted with asphalt and sunk into the ground on a well drained sandy knoll. The soil in these containers was also sampled for chemical analysis over the 21-months' weathering period.

On each sampling date, sufficient cores, 1×6 inches, were taken with a 1-inch soil tube to fill a 1-quart Mason jar from each treatment. These samples were also stored in the 40° F. cold room pending chemical analysis.

Results.—The amounts of heptachlor persisting in the soil in the field plots and the galvanized cylinders are summarized in table 2. the results of this experiment were disappointing, for the variance involved in the residue determinations was too great to permit the detection of the influence of soil type. However, certain general trends are suggested by the data. Twenty-one months after the treatments were applied, approximately 26% of the heptachlor was still present in the field plots, while 53% persisted in the soil in the metal cylinders. These are averaged figures over all soil types. It is reasonable to assume that the insecticide persisted in greater amounts in the cylinders, because it was more thoroughly mixed

Table 2.—The persistence of heptachlor residues in field soil plots and laboratory treated soil placed in galvanized metal cylinders and exposed to the weather for a period of 21 months.

Soil Type	Calculated Amount Applied (Lb./6 Inch Acre)	Residue Persisting at Four Time Intervals of Weathering (Months) (Lb./6 Inch Acre)				Per Cent Persisting[a] After 21 Months
		0	4	12	21	
Field Plots[b]						
Sandy Loam	2	1.08	0.58	0.50	0.45	42
	4	2.21	1.59	1.06	.83	38
Silty Clay Loam	2	1.22	—	.28	.33	27
	4	2.84	—	.64	.48	17
Muck	2	3.35	.75	.74	.50	15
	4	4.34	1.46	1.34	.74	17
Mean per cent persisting for all soil types in field plots						26
Metal Cylinders[c]						
Sandy Loam	2	0.68	1.07	0.84	0.55	81
	4	1.87	2.00	1.79	.90	48
Silty Clay Loam	2	1.21	.83	.81	.39	32
	4	2.11	1.63	1.74	.96	45
Muck	2	.94	.51	.50	.38	40
	4	1.94	1.86	1.79	1.35	70
Mean per cent persisting for all soil types in metal cylinders						53

[a] Based on the amount detected by chemical analysis at 0 time.
[b] Each entry represents the average value from two replicate plots. Standard error of a mean = .39.
[c] Each entry represents a single determination. Standard error of a single determination = .14.

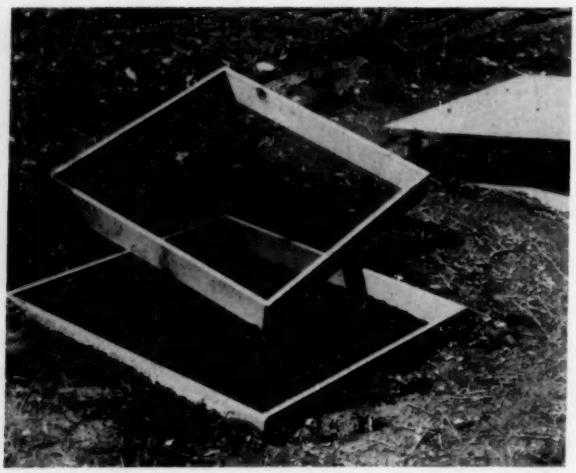


FIG. 1.—A frame used in the spray residue studies shown with the lower soil layer in place. The adjacent frame with sun shield is also visible.

to a greater depth than was the case in the field.

The discrepancies between the calculated amount applied and that detected chemically at zero weathering time cannot be completely accounted for. This discrepancy cannot be attributed to incomplete recoveries in the extraction procedure, for re-extraction of several of the samples indicated that recoveries on the first extraction were near 100%

INSECTICIDE APPLIED ON THE SOIL SURFACE.—The second phase of this problem was concerned with the rate of accumulation and persistence of heptachlor residues that might result from spray reaching the soil in connection with the application of this material to the foliage of plants.

1953 Experiment.—This experiment was designed to study the rate of accumulation of heptachlor in muck soil from a series of spray applications applied to the soil surface. The Oswego County muck used was sifted through a ¼-inch screen and placed in three double wooden frames, 36 inches square, and 8 inches deep and exposed to the weather in the field. These frames were so constructed that a top 3-inch layer of soil was separated from the 5-inch layer below it by a 16-mesh copper screen. The construction of one of these frames is shown in figure 1. The two soil layers could therefore be sampled separately to determine the extent of downward leaching of heptachlor through the soil.

Six weekly applications of heptachlor, from the emulsion formulation, were made at the rate of 0.33 pound of actual material per acre to the surface in all the frames. One frame was exposed to the weather at all times, another was shaded from the sun by a hardboard (Masonite) shield painted with aluminum to reduce the effect of solar radiation. The latter simulated the effect of a foliage cover by shielding the soil from the direct rays of the sun. The third frame was exposed to the weather and to increase the possibility of leaching was watered with distilled water in amounts equivalent to the natural rainfall that occurred (3.2 inches) during the spray period. The shaded frame was watered with distilled water in amounts equivalent to the natural rainfall. These treatments were designated "sun," "shade," and "water," respectively.

The spray applications were made with a small carbon dioxide pressurized sprayer consisting of a CO_2 cylinder, a pressure regulating valve that regulated the pressure at 30 pounds per square inch and a milk bottle tank equipped with a shut-off valve connected with a rubber hose to Tee-Jet nozzle (No. SS8001). One hundred milliliters of spray was applied to the soil surface in each frame on each of the six application dates. The first spray was applied on July 31 and the last on September 10. A sample of untreated soil was taken from each frame before the first spray was applied. Another sample was taken for analysis, from the "sun" frame immediately after the first application, to compare the determination with the calculated amount applied. Before each of the following weekly sprays were applied, the upper soil layer in each frame was sampled for chemical analysis of the residue present on that date. These samples were taken with the soil sampler set to take 2-inch cores 2 inches in depth. Enough cores were taken from each frame to fill a 1-quart Mason jar. After each sample was taken the soil surface was leveled with a rake. One week after the sixth spray both upper and lower layers of soil in the three frames were sampled as described above. The upper frames were returned to position and the soil was exposed to the weather for the winter months. The following June, both soil layers were again sampled to determine the quantity of heptachlor that had persisted through the winter. All samples collected were air dried, extracted and analyzed chemically for heptachlor content.

Results.—The results of the experiment are summarized in table 3. Although heptachlor did accumulate in the soil during the 6-week spray period, 1 week after the last application only 30% of the total amount that had been applied was still present in the upper soil layer in each of the three frames. No heptachlor was present in the three lower soil layers on the same date. The following summer no heptachlor was detected in either the upper or lower soil layers. The accumulation of heptachlor was esentially the same in the "sun," "shade" and "water" treatments, although slightly more persisted in the "water" soil surface for the first 5 weeks of the experiment.

Table 3.—The residue of heptachlor in muck soil resulting from 6 weekly applications to soil surfaces at 0.33 pound per acre and subjected to three conditions of weathering.

Sampling Date	Calculated Deposit Present (Lb./2-inch Acre)	Residue Present (Lb./2 Inch Acre)		
		Upper Soil Layer		
		Sun	Shade	Water
July 31				
Before treating	0	0	0	0
After treating	.33	.27	—	—
August 7	.33	.13	.10	.18
August 14	.66	.14	.17	.26
August 21	1.00	.28	.26	.45
August 28	1.33	.24	.30	.34
September 4	1.66	.45	.42	.42
September 10	2.00	.60/31[a]	.62/30	.60/30
June 15	2.00	0	0	0

[a] Per cent of the total amount applied persisting 1 week after the last application.

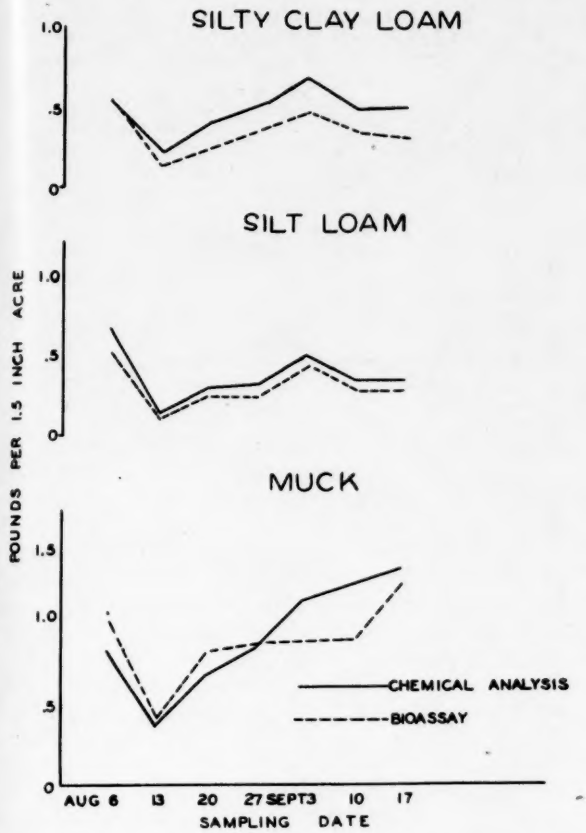


FIG. 2.—The rates of accumulation of heptachlor residues in soils of three types, as determined with a specific colorimetric chemical analysis of soil extracts and a bioassay of the soil directly with *Drosophila*.

These results indicate that heptachlor will not accumulate to harmful levels in cultivated muck soils where a total of 2 pounds has been applied in a series of six weekly sprays.

The 1954 Experiment.—During the summer of 1954 the spray residue studies were continued and expanded to study the effects of soil type, insecticide formulation, and cultivation upon the rate of accumulation of heptachlor from a series of six sprays applied weekly to the soil surface.

Three soil types were included in this experiment; Dunkirk silt loam, Dunkirk silty clay loam and muck. The two mineral soils were present in the Experiment Station field, and the muck was obtained from Oswego County.

Wooden frames of construction similar to those used the previous year were placed in the field. Eight frames or plots for each of the three soil types were provided and upon these eight plots the following treatments were superimposed in a factorial arrangement. Four received heptachlor from emulsion formulation and four from a wettable powder. Four were shaded with hardboard shields and four were exposed to the sun. Four were cultivated and four were undisturbed throughout the experiment.

Six sprays of heptachlor were applied at weekly intervals to all soil surfaces at the rate of 0.7 pound per acre per application. These sprays were applied with the same sprayer as used in the experiment of the previous year. One hundred millilters of spray was applied per frame with each application. The first spray was applied August 6 and the last on September 17.

Soil samples from four boxes of each soil type selected at random were taken before the first spray was applied to be used as untreated checks. Immediately after the first spray had been applied, the four frames of each soil type that had been assigned the cultivation treatments were sampled. This set of samples provided an estimate of the experimental error that was associated with the determination of the residue present. If no error had been involved, the amount of heptachlor detected in all frames at this time should have been the same.

The cultivated frames were sampled and then cultivated with the hand rake prior to each successive application. The top soil layer in all frames, both cultivated and undisturbed, was sampled 1 week after the last application. Both upper and lower layers of all frames were sampled the following spring on April 2. All samples were taken according to the procedure described for the 1953 experiment, except the sampling depth was reduced to 1.5 inches.

The temperatures at the surface of the soil and 3 inches below the surface were recorded for a 1-week period for each of the soil types. During the 6-week spray period, 5.2

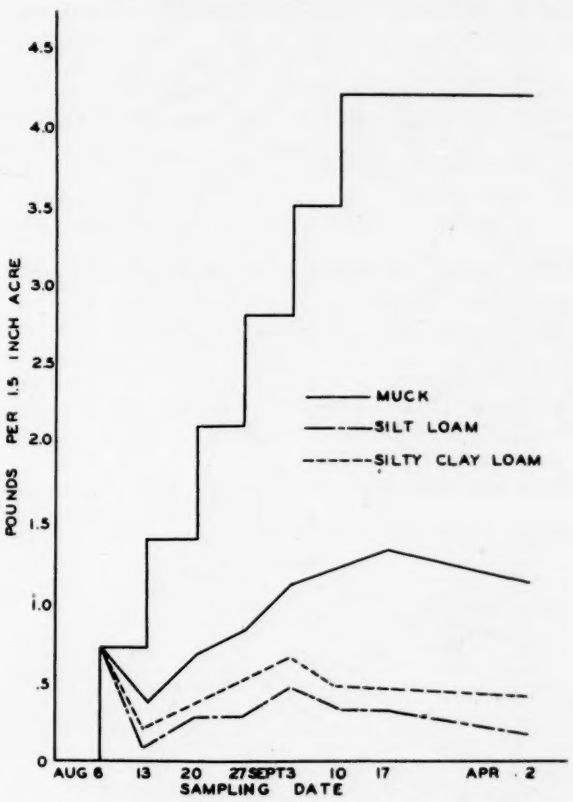


FIG. 3.—The rate of accumulation of heptachlor in three types of soil, resulting from six weekly applications of 0.7 pound per acre to the soil surface. The step curve indicates the calculated amount of insecticide applied.

inches of natural rainfall occurred. During most of this rain, the sun shields were removed. When this was not possible distilled water was added to the shaded soil surfaces in a amount equivalent to the natural rainfall that occurred. The shades were removed from the frames on September 17 for the remainder of the fall and winter.

All the soil samples collected through September 17 were analyzed for the heptachlor present with both the chemical and biological methods of analysis. At the time the samples were air dried, prior to chemical analysis, 20-gram subsamples of the muck and 30-gram subsamples of the mineral soils were set aside for the bioassay with *Drosophila*. Thus a total of 96 samples were analyzed for heptachlor residues by both methods. The samples taken on April 2 were analyzed by the chemical method only.

Results and Conclusions.—The residues of heptachlor, as determined chemically and biologically, are summarized for the three soil types in table 4, and are presented graphically in figure 2. Differences between the results obtained with the two methods were tested for significance with a "t" test using paired observations according to the procedure described by Youden (1951). The results obtained with the two methods on the muck soil were not significantly different. A significantly greater amount was detected in both mineral soils with the chemical analysis than with the bioassay. It appears, therefore, that

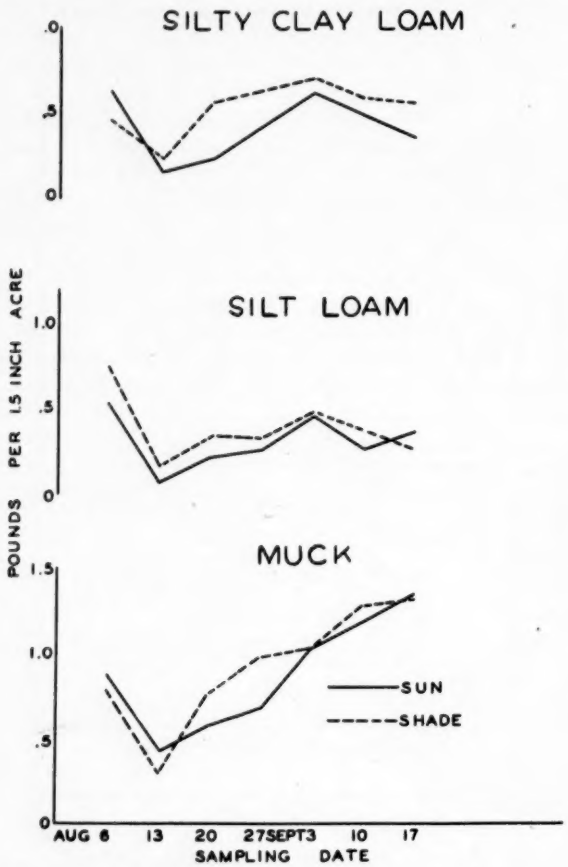


FIG. 4.—The effect of exposure to the sun, upon the rate of accumulation of heptachlor in three soil types, resulting from six weekly spray applications of 0.7 pound per acre.

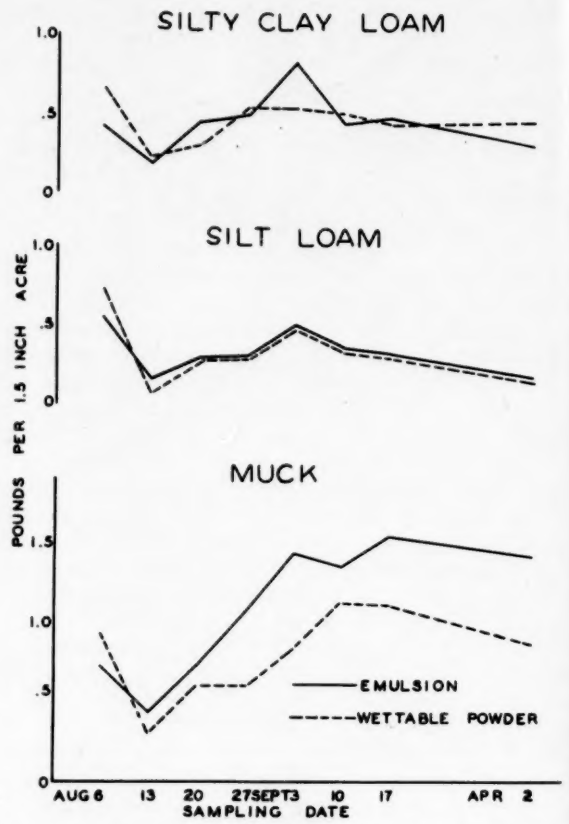


FIG. 5.—The interaction of the effects of soil type and insecticide formulation upon the rate of accumulation of heptachlor in soils, resulting from six weekly spray applications to the soil surface of 0.7 pound per acre.

the extraction procedure used for the chemical analysis was able to recover more heptachlor from these soils than was available to the test insects in the bioassay. Since the residues determined chemically were equal to or greater than those determined biologically, there was no evidence that toxic degradation products or metabolites of heptachlor were present in the soils.

As all the samples involved in this experiment were analyzed by the chemical method, the following discussion of the effects of the treatments applied is based upon the data obtained by this method of analysis. The residues of heptachlor that accumulated in the soil as influenced by the effects of formulation, soil type, sun exposure and cultivation are summarized in table 5. The data for the six sampling dates were analyzed using a split plot factorial analysis of variance. One week after the last application, 31% of the total amount of heptachlor that had been applied was still present in the muck soil, while 8% persisted in the silt loam and 11% was still present in the silty clay loam. These differences between soil types illustrated graphically in figure 3, were highly significant. The rates of accumulation appeared to be correlated with the adsorptive capacities of the three soil types. The amount of residual heptachlor in the muck soil continued to increase after the fifth and sixth applications, while it reached a maximum after the fourth spray in the mineral soils. This accounts for the significant interaction of

Table 4.—The residue of heptachlor in soils of three types resulting from six weekly sprays applied to the soil surface of 0.7 pound per acre as determined by chemical analysis and Drosophila bioassay.

Sampling Date	Calculated Deposit Present (Lb./1.5 Inch Acre)	Residue Persisting (Lb /1.5 Inch Acre)					
		Muck		Silt Loam		Silty Clay Loam	
		Chemical	Bioassay	Chemical	Bioassay	Chemical	Bioassay
Aug. 6	0.7	0.84[a]	1.02	0.65	0.52	0.53	0.54
13	.7	.36	.41	.13	.10	.21	.13
20	1.4	.66	.82	.28	.23	.38	.24
27	2.1	.84	.86	.30	.23	.51	.36
Sept. 3	2.8	1.12	.86	.48	.42	.67	.44
10	3.5	1.23	.88	.34	.28	.48	.32
17	4.2	1.32	1.22	.32	.26	.47	.28

[a] Each entry is the average of four determinations, one from each of the cultivated frames.

soil types with sampling dates in the analysis of variance. This interaction is apparent in figure 3.

Although the difference in accumulation rate between the shaded and exposed soil surfaces was not significant at the 5% level of probability, there was a definite indication shown in figure 4 that more heptachlor accumulated in the shaded soil. The mean maximum temperatures of the exposed soils were as much as 25° F. higher than in the shaded soils in some cases, which may account for the greater losses from the soils exposed to the sun.

Heptachlor applied as an emulsion persisted in quantities significantly greater than that applied as a wettable powder. This effect of formulation was greatest with the muck soil and less in the case of the silty clay loam. This interaction is shown in figure 5.

Heptachlor persisted in equal amounts in the cultivated and undisturbed soil surfaces. It is possible that the superficial cultivation applied in this experiment did not carry the insecticide as deeply into the soil as might be the case in actual field practice.

No heptachlor was detected in the lower soil layers on April 2, indicating that none of the insecticide that had disappeared from the upper layer had been leached to the soil below the screen.

General Discussion and Conclusions.—From the results of this study, it does not appear that harmful accumulations of heptachlor in cultivated soils will result from application of this insecticide for the control of foliage pests. Although a considerable amount of the toxicant in these sprays may reach the soil, its loss from the soil surface is quite rapid. This rate of loss is influenced by soil type with the most rapid losses from coarse textured soils low in organic matter and adsorptive properties.

Where heptachlor may be mixed with the soil, in treatments for the control of soil inhabiting insects, it will persist for longer periods.

There is no evidence to indicate that the losses of heptachlor from the soil may be due to leaching. If this compound does decompose or is metabolized by soil microorganisms the degradation products or metabolites are not toxic to *Drosophila melanogaster*.

Summary.—The persistence of heptachlor in four soil types, Dunkirk sandy loam, Dunkirk silt loam, Dunkirk silty clay loam and muck, was determined.

Where heptachlor was incorporated in the soil of field plots at 2 and 4 pounds per acre, an average of 26% of the amount present after treatment persisted for at least 21 months. When heptachlor was thoroughly mixed in the laboratory into soils of the same types at the rates of 2 and 4 pounds per acre, and this treated soil was exposed to the weather in metal cylinders placed in the ground, an average of 53% of the amount applied persisted for at least 21 months. Differences in persistence of heptachlor in the different soil types were non-significant due to wide variability among replicates.

In studies involving six weekly spray applications to the soil surface only 31% of the total amount applied was present 1 week after the last application in the muck soil.

Table 5.—The residues of heptachlor resulting from a series of six weekly spray applications to the soil surface of 0.7 pound per acre, as affected by formulation, type of soil, exposure to the sun and cultivation.

Sampling Date	Calculated Deposit Present (Lb./1.5 Inch Acre)	Residue Persisting (Lb./1.5 Inch Acre)								
		By Formulation		By Soil Type			By Sun Exposure		By Cultivation	
		W.P.	Emul.	Muck	Silt Loam	Silty Clay Loam	Sun	Shade	Cultivated	Undisturbed
Aug. 6	0.7	0.94	0.74	0.84	0.63	0.53	0.89	0.79	—	—
13	.7	.20	.26	.36	.13	.21	.23	.23	—	—
20	1.4	.40	.48	.66	.28	.38	.34	.55	—	—
27	2.1	.48	.62	.84	.30	.51	.45	.64	—	—
Sept. 3	2.8	.61	.90	1.11	.48	.67	.74	.77	—	—
10	3.5	.64	.72	1.23	.34	.48	.64	.72	—	—
21	4.2	.62/15[a]	.79/19	1.32/31	.32/8	.47/11	.69/16	.72/17	0.70/17	0.73/17
April 2	4.2	.51	.65	1.14	.19	.40	—	—	.14	.11

[a] Per cent of the total amount applied persisting 1 week after the last spray.

Under similar conditions 11% persisted in a silty clay loam and 8% persisted in a silt loam. Losses of heptachlor from the soil were less from an emulsion formulation than from a wettable powder. Losses of this insecticide from all soil types were greater from soil surfaces exposed to the sun than from shaded soil surfaces. Superficial cultivation of the soil surface during the spray period apparently did not affect the persistence of this insecticide. No evidence that heptachlor is leached from the soil or is broken down into toxic degradation products or metabolites, was found in this investigation.

The conclusion is drawn that heptachlor will not accumulate to dangerous levels in cultivated soils when applied as a soil treatment or as a foliage spray in the amounts recommended for efficient insect control.

REFERENCES CITED

Bigger, J. H. 1955. A report given at Spray Operators School, Urbana, Ill. January 20–21, Agric. Chemicals **10**: 122.

Buoyoucos, G. J. 1936. Directions for making mechanical analysis of soils by the hydrometer method. Soil Sci. **42**: 225–30.

Fleming, W. E. 1950. Persistence of effect of DDT on Japanese beetle larvae in New Jersey soils. Jour. Econ. Ent. **43**(1): 87–9.

Fleming, W. E., and W. W. Maines. 1953. Persistence of DDT in soils of the area infested by the Japanese beetle. Jour. Econ. Ent. **46**(3): 445–9.

Fleming, W. E., and W. W. Maines. 1954. Persistence of chlordane in soils of the area infested by the Japanese beetle. Jour. Econ. Ent. **47**(1): 165–9.

Johnson, V. A., and W. L. Caskey. 1952. Soil Sampler. U. S. Dept. Agric. Bur. Ent. and Plant Quar. **ET-298.**

Kirk, V. M. 1952. A study of the disposition of DDT when used as an insecticide for potatoes. Cornell University Agric. Expt. Sta. Mem. **312.**

Koblitsky, L., and R. D. Chisholm. 1949. Determination of DDT in soils. Jour. Assoc. Offic. Agric. Chemists **31**: 781–6.

Olson, R. E. 1954. The use of the fruit fly, *Drosophila melanogaster*, as a bioassay in detecting minute quantities of benzene hexachloride in plant tissue. Unpublished Ph.D. Thesis, Cornell University.

Ordas, E. P., V. C. Smith, and C. F. Meyer. 1954. Spectrophotometric analysis of technical chlordane and technical heptachlor on food and forage crops. A paper presented before the 126th National Meeting American Chemical Soc., New York, N. Y. September.

Polen, P. B., and P. Silverman. 1952. Detection and determination of 1, 4, 5, 6, 7, 8, 8, heptachlor-3a, 4, 7, 7a. tetrahydro-4, 7-methanoindene. Analyt. Chem. **24**: 733–5.

Terriere, L. C., and D. W. Ingalsbe. 1953. Translocation and residual action of soil insecticides. Jour. Econ. Ent. **46**(5): 751–3.

Youden, W. J. 1951. Statistical Methods for Chemists. John Wiley and Sons Inc., New York, 126 pp.

A Field Strain of Malathion-Resistant House Flies[1]

JOHN W. KILPATRICK and H. F. SCHOOF

Insect resistance can be defined as the ability of certain strains of a species to survive, by one or more mechanisms, exposure to insecticides which previously had produced mortalities at the same dosages. In the field, resistance has been principally of the physiologic type and confined to the chlorinated hydrocarbon insecticides. However, recently, Keiding (1956) reported significant field resistance in Denmark by house flies to Bayer 21/199 (Resitox). House fly resistance to organic phosphorus compounds has not been observed previously in the United States, even though several such pesticides have been in common usage since 1954. This report presents data on both physiologic and behavioristic resistance to malathion in a field strain of house flies in the Savannah, Georgia, area.

During 1954 and 1955 (Kilpatrick & Schoof 1957) dairies "Be" and "Ro" received residual treatments of malathion (plus sugar) at a dosage of 200 mg /sq. ft., the former being sprayed three times, the latter twice. Dairy "Be" also received an application of Dipterex (plus sugar) at 100 mg./sq. ft., while dairy "Ro" was given routine malathion bait treatments in the latter part of 1955. To evaluate the efficiency of poison baits applied on a routine schedule at permanent bait stations, studies were conducted at these dairies in 1956 wherein the baits were renewed twice weekly to reduce fly populations to control levels and once per week thereafter. A commercial bait containing 2% malathion was dispensed in plywood trays (2′×2′) at 12 to 16 sites of high fly concentrations outside the milking barn, at a dosage of 4 ounces of bait per 1,000 sq. ft. of floor area.

House fly populations at dairy "Be" were reduced to excellent control levels immediately (grill index at or below 10 flies) and only a single application per week was required after the second post-treatment week (table 1). At dairy "Ro" excellent control was not obtained as rapidly and twice-weekly treatments were continued for 3 weeks. Satisfactory control (grill index at or below 20 flies) was not obtained at dairy "Ro" until permanent bait stations (4″×12″) also were established in the milking barn. After such installation, excellent control was obtained at this dairy for the remainder of the fly season except for 1 week. Cessation of treatment for a 2-week period allowed a slight increase in the fly population.

Excellent control was maintained at dairy "Be" from mid-May until the first of August. At this point fly populations began to increase despite a reversion to twice-weekly treatments (table 1). Treatments with other type malathion bait formulations likewise failed to provide fly control. Observations and specific tests showed that flies at diary "Be" were not attracted to any of the mala-

[1] From the Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia, Accepted for publication July 8, 1957.

Table 1.—House fly grill counts in dairy barns near Savannah, Georgia, treated weekly with malathion poison baits at the rate of 4 ounces of bait per 1,000 square feet of floor area. 1956.

Weeks After Treatment	Dairy "Be"	Dairy "Ro"
−3	11	21
−2	89	34
−1	159	216
0	406	194
Initial Treatment	May 21	May 14
1	9[a]	34[a]
2	5[a]	19[a]
3	4	10[a]
4	3	8
5	4	35
6	5	30
7	9	26[b]
8	4	13
9	7	6
10	11	8
11	16	10
12	17	8
13	70[c]	8[c]
14	120	9[c]
15	59[a]	17
16	62[a]	4
17	170	
17	165[d]	7
17	97[e]	
17	58	
18	85	4
19	70	3
20	62	5
21	77	5

[a] Two treatments per week.
[a] Bait stations added to milking barn.
[c] No treatment.
[d] Treated with purina malathion fly bait.
[e] Treated with 2 gallons of 2% malathion-10% sugar liquid bait.
[f] Treated with malathion fly flakes.

thion bait formulations and even appeared to be repelled by them (table 2). A majority of the specimens now approached the bait in flight but failed to alight on it, a reaction in contrast to the previous response. However, these flies showed no aversion to landing and feeding upon Dipterex or DDVP baits. The same malathion baits were evaluated at other dairies around Savannah, Georgia, (table 3) and in a few locations near Orlando, Florida, and were found to be highly attractive to house flies at all these sites.

Differences in the responses of house flies from dairy "Be" and of a susceptible NAIDM (National Association of Insecticide and Disinfectant Manufacturers) strain to malathion and Dipterex baits were shown in the laboratory (Fay *et al.* 1957). Their data revealed that flies from dairy "Be" were not attracted to the malathion bait formulations tested whereas the NAIDM strain accepted the baits. Both strains accepted a Dipterex bait.

Table 2.—Number of house flies attracted to baits at dairies "Be" and "Ro" September 14, 1956.

Minutes After Bait Exposure	2% Malathion[a]		2% Malathion :10% Sugar[b]		1% DDVP :10% Sugar[b]	
	"Be"	"Ro"	"Be"	"Ro"	"Be"	"Ro"
1	0	27	0	5	52	10
2	0	13	0	3	75	10
3	0	15	0	3	>200	11
4	0	15	0	4	>200	11
5	0	14	0	2	>200	2

[a] Commercial bait A, dry.
[b] Experimental bait, liquid.

Table 3.—Number of house flies attracted to five commercial dry baits at dairies "Be" and "Z", September 21, 1956.

Minutes After Bait Exposure	Malathion Formulation 1		2		3		4		Dipterex	
	"Be"	"Z"	"Be"	"Z"	"Be"	"Z"	"Be"	"Z"	"Be"	"Z"
10	10	92	9	38	11	62	1	16	>300	124
20[a]	8	40	10	30	9	14	1	9	>500	91
30	7	36	4	13	7	15	1	8	—	—

[a] Decrease in the number of flies on baits at dairy "Z" was due to mortalities of the fly populations.

Further laboratory tests (Fay *et al.*1957) also showed that the "Be" strain of flies was less susceptible to malathion-sugar deposits on plywood panels than was the NAIDM strain. In addition, topical applications of a malathion-acetone solution on 3-day-old adult females of the NAIDM and "Be" strains gave LD-50 values of 0.5 and 1.2 micrograms per fly, respectively. A dosage of 1 microgram per fly gave 100% kill of the NAIDM strain in contrast to 7 micrograms per fly for the "Be" strain. These tests indicate a degree of physiologic resistance in the field strain.

Thus the data for the response of house flies in dairy "Be" to malathion baits reveal this strain to have a dual type of resistance to malathion. Presumably the behavioristic phase was the chief factor responsible for the loss of control.

References Cited

Keiding, J. 1956. Resistance to organic insecticides of the house fly. Science **123**(3209): 1173–4.

Fay, R. W., John W. Kilpatrick, and G. Clinton Morris, 1957. Malathion resistance studies on the house fly. In manuscript.

Kilpatrick, John W., and H. F. Schoof. 1957. House fly control in dairy barns with residual treatments of phosphorus compounds. Jour. Econ. Ent. **50**(1): 36–9.

Organic Phosphorus Compounds for the Control of Resistant House Flies in Dairy Barns[1]

H. F. Schoof and John W. Kilpatrick

Extensive field tests using residual treatments of various organic phosphorus compounds for control of resistant house flies have been reported by Hansens *et al.* 1955a and 1955b, Dahm & Raun 1955, Gersdorff *et al.* 1955, Hansens 1956, DeFoliart 1956, Goodwin *et al.* 1956, and Kilpatrick & Schoof 1957. Results of these tests varied greatly, depending on geographic location, method of application and evaluation, and formulation used. In general, control was achieved for 3 to 6 weeks, but individual test effectiveness ranged from 1 to 9 weeks.

In 1956, further tests were made on the effectiveness of organophosphorus compounds as residual treatments for house fly control in the Savannah, Georgia, area.

Methods.—Dairies in the Savannah milk shed have herds of 100 to 400 animals. Sanitation is relatively poor at all dairies but fly potential at each varies depending on the condition of the herd, the moisture content of the manure, the number of calves penned, and other factors.

Insecticides were applied as residual treatments to the interior surfaces of the milking barn, calf pens, and other animal shelters at 100 p.s.i. by a power sprayer equipped with flat spray nozzles. Materials tested included Diazinon at 20 and 100 mg./sq. ft. with and without a sugar additive, Dipterex at 100 mg./sq. ft. with and without sugar, Dow-ET-15 at 200 mg./sq. ft., DDT:EPN at 200:25 mg./sq. ft., DDT:parathion at 200:10 mg./sq. ft., parathion:sugar at 10:250 mg./sq. ft., and DDVP:Aroclor at 100:400 mg./sq. ft. All formulations were water emulsions except Dipterex (water solution) and DDT:EPN (suspension). Application was at a rate of 1 gallon of finished spray per 1,000 sq. ft.

The efficacy of the residual treatments was determined by making weekly grill counts of the house flies at each

[1] From the Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia. Accepted for publication July 8, 1957.

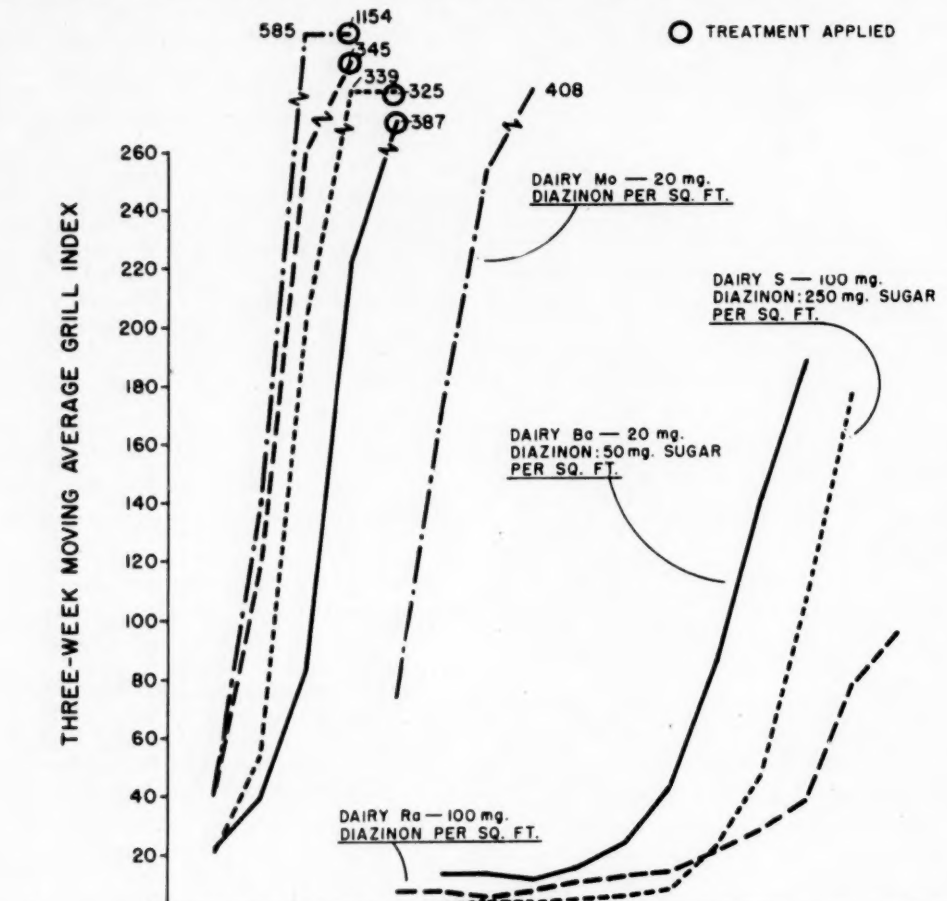


Fig. 1.—House fly control in dairy barns near Savannah, Georgia, treated with residual applications of Diazinon emulsions, 1956.

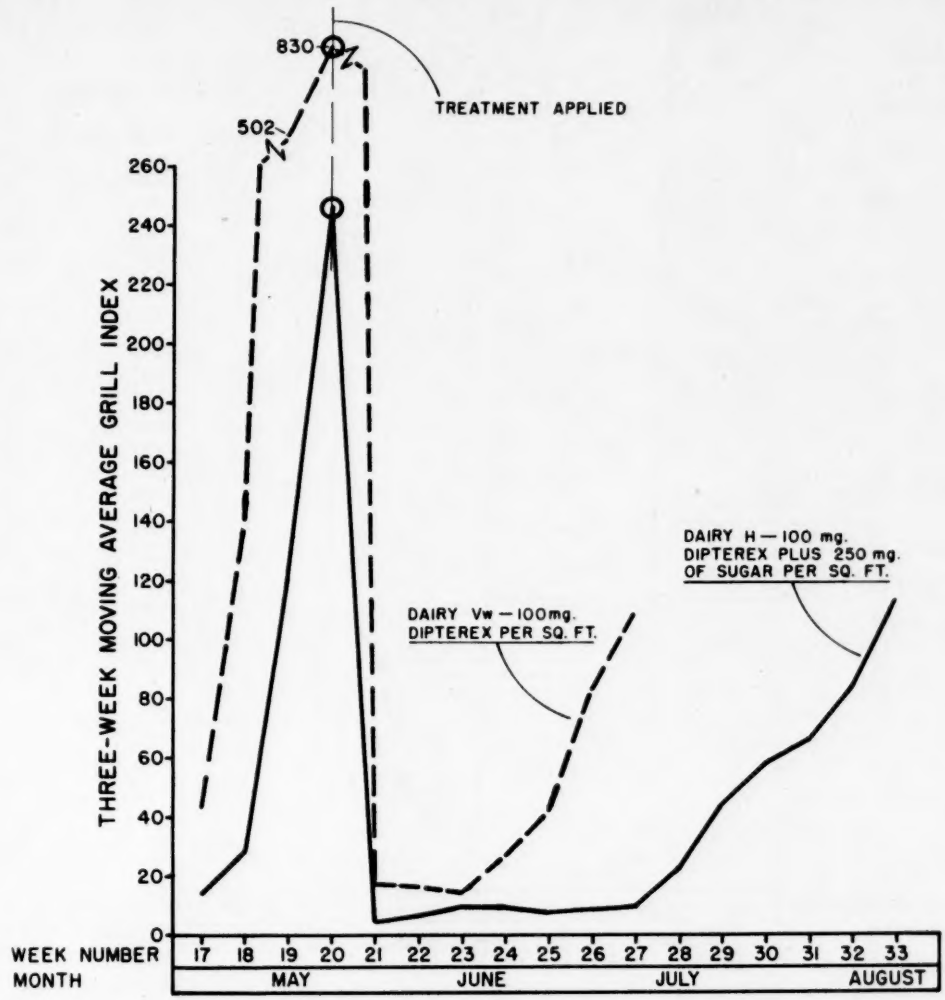


FIG. 2.—House fly control in dairy barns near Savannah, Georgia, treated with residual applications of Dipterex solutions, 1956.

dairy. Five counts were made at each of four general locations (stanchions, building entrances, barn floor, and feed room) known to be attractive to high fly populations. The fly index for a dairy is the average of four maximum counts, one from each of the four locations. Indices below 10 were considered as representing excellent control; those below 20 as satisfactory control.

RESULTS.—Two dairies were treated with Diazinon at dosages of 20 (Dairy "Ba") and 100 (Dairy "S") mg./sq. ft. with an additive of $2\frac{1}{2}$ parts of sugar to 1 part insecticide. Two additional dairies received treatments of Diazinon alone (Dairy "Mo," 20 mg./sq. ft., Dairy "Ra" 100 mg./sq. ft.). At all four dairies, pretreatment fly indices were at extremely high levels (fig. 1) but treatment in the latter part of May caused immediate reductions in each dairy. Excellent house fly control was obtained at dairy "Ra" (without sugar) for 4 weeks with an additional 4 weeks of satisfactory control. At dairy "S" (with sugar), 6 weeks of excellent control was obtained with rapidly rising indices noted beyond this period. From the practical aspect, the addition of sugar to the Diazinon treatment of 100 mg./sq. ft. did not improve results materially.

Diazinon emulsions applied at a dosage of 20 mg./sq. ft. did not provide control comparable with results achieved at dairies treated at a dosage of 100 mg./sq. ft. Satisfactory control resulted at dairy "Ba" (with sugar) for a period of 4 weeks whereas at dairy "Mo" (without sugar) unsatisfactory fly indices occurred during the first post-treatment week. Thus it appears that the addition of sugar to this dosage of Diazinon markedly increases its residual efficacy.

At dairy "H" Dipterex:sugar at 100:250 mg./sq. ft. gave 8 weeks of excellent to satisfactory fly control (fig. 2) confirming previous field results (Kilpatrick & Schoof 1957). However, at dairy "VW" treated with Dipterex alone at 100 mg./sq. ft., only 3 weeks of satisfactory control was achieved, thus indicating the value of a sugar additive.

Unpublished laboratory data indicate the residual action of parathion against house flies resistant to chlorinated hydrocarbon insecticides is moderately improved by the addition of DDT. Three dairies treated at a dosage of 10 mg. parathion:200 mg. DDT gave varying results: 7 weeks of satisfactory to excellent control at dairy "Rg"; only 1 and 5 weeks of satisfactory control at dairies "Mo"

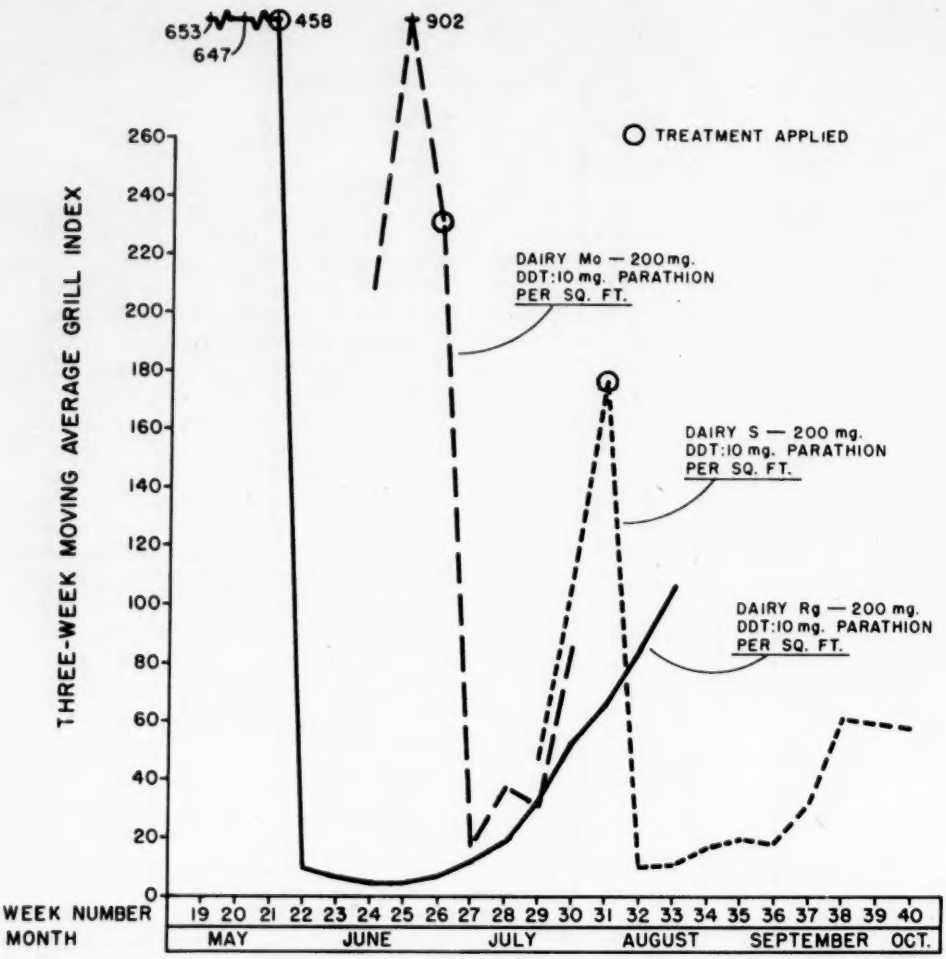


FIG. 3.—House fly control in dairy barns near Savannah, Georgia, treated with residual applications of DDT:parathion emulsions, 1956.

and "S" respectively (fig. 3). A fourth dairy ("VW") sprayed with a parathion:sugar formulation of 10 mg. parathion:250 mg. sugar/sq. ft. yielded a single week of excellent control. The erratic performance of these treatments, together with its high mammalian toxicity, indicates that parathion has little promise as a residual spray treatment in house fly control.

Similar laboratory data also showed that EPN:DDT combinations were effective against resistant house flies. An EPN:DDT suspension applied at dairy "W" at the rate of 25 mg. EPN:200 mg. DDT/sq. ft. gave immediate reduction of the fly population to an excellent control level which was maintained for the remainder of the 13-week test period (table 1). Wall cage tests using 30-minute exposures conducted approximately 3 months after treatment gave an average of 70% mortality of DDT-resistant flies.

The application of Dow-ET-15 at Dairy "Ba" at the rate of 200 mg./sq. ft. yielded excellent control which continued for the entire 9-week test period (table 1). Cool weather precluded further observations, with future tests indicated to fully assay the residual effectiveness of this compound.

Laboratory data presented by Hornstein *et al.* (1955) indicated that improved results might be obtained from a DDVP:Aroclor-5460 combination when applied at a 1:4 ratio as a residual treatment. DDVP-Aroclor-1256 when used in one dairy barn at the rate of 100:400 mg./sq. ft. produced excellent control for 2 weeks. However, flies were observed resting on sprayed surfaces for 1 hour or more only a few days after treatment without knockdown, indicating that control was largely due to space spray action during the application. After 2 weeks, the owner began using poison baits because of rising fly populations.

SUMMARY.—At Savannah, Georgia, in 1956, residual applications of Diazinon to dairy barns at a rate of 100 mg./sq. ft. alone or with sugar (250 mg./sq. ft.) gave 6 to 8 weeks of satisfactory control. At the rate of 20 mg./sq. ft., Diazinon without sugar failed to provide control but with sugar (50 mg./sq. ft.) produced 4 weeks of satisfactory reduction.

A Dipterex-water solution applied at the rate of 100 mg./sq. ft. gave 3 weeks of satisfactory control compared with 8 weeks of control obtained with a Dipterex:sugar formulation (100:250 mg./sq. ft.).

Table 1.—House fly control in dairy barns near Savannah, Georgia, with DDT:EPN and Dow-ET-15 residual sprays, 1956.

Weeks After Treatment	Grill Index—3-Wk. Moving Average	
	Dairy "W" DDT: EPN Suspension (200:25 Mg./Sq. Ft.)	Dairy "Ba" Dow-ET-15 Emulsion (200 Mg./Sq. Ft.)
−1	79	140
−0	78[a]	187[b]
1	2	3
2	2	2
3	1	3
4	3	3
5	5	3
6	5	4
7	5	5
8	4	4
9	3	6
10	3	
11	3	
12	4	
13	4	

[a] Treated July 9, 1956.
[b] Treated August 1, 1956.

Parathion:DDT emulsion formulations at the rate of 10:200 mg./sq. ft. gave control ranging from 1 to 7 weeks. A parathion:sugar emulsion treatment at the rate of 10:250 mg./sq. ft. produced only 1 week of excellent control.

A single test with an EPN:DDT suspension at a rate of 25:200 mg./sq. ft. gave excellent control for the entire 13-week evaluation period. Dow ET-15 emulsion applied late in the season at the rate of 200 mg./sq. ft. provided excellent control for the 9-week evaluation period.

References Cited

Dahm, Paul A., and E. S. Raun. 1955. Fly control on farms with several organic thiophosphate insecticides. Jour. Econ. Ent. **48**(3): 317–22.

DeFoliart, Gene R. 1956. Fly control in Wyoming barns. Jour. Econ. Ent. **49**(3): 341–4.

Goodwin, W. J., and F. R. Gressette. Jr. 1956. Residual house fly control in dairy and beef barns in South Carolina. Jour. Econ. Ent. **49**(5): 622–4.

Hansens, Elton J., Philip Granett, and Charles T.O'Conner. 1955. Fly control in dairy barns in 1954. Jour. Econ. Ent. **48**(3): 306–10.

Hansens, Eiton J., and Roger Scott. 1955. Diazinon and Pirazinon in fly control. Jour. Econ. Ent. **48**(3): 337–8.

Hansens, Elton J. 1956. Control of house flies in dairy barns with special reference to Diazinon. Jour. Econ. Ent. **49**(1): 27–32.

Hornstein, Irwin, W. N. Sullivan, and Ching Hsi Tsao. 1955. Residual effectiveness of mixtures of organic phosphorus insecticides with chlorinated terphenyls. Jour. Econ. Ent. **48**(4): 482–3.

Kilpatrick, J. W., and H. F. Schoof. 1957. House fly control in dairy barns with residual treatments of phosphorus compounds. Jour. Econ. Ent. **50**(1): 36–9.

Some Factors Influencing the Infestation in Corn in the Field by the Rice Weevil[1]

E. H. Floyd and J. D. Powell, *Louisiana Agricultural Experiment Station, Baton Rouge,* and J. W. Ingram, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

The value of good husk cover of corn in the alleviation of damage by the rice weevil, *Sitophilus oryza* (L.), has been demonstrated by a number of workers (Hinds 1914, Kyle 1918, Back 1919, Cartwright 1936, Massop 1940, Eden 1952 and others). Cartwright showed that the length of the shuck extension beyond the tip of the ear was an important factor in the development of insect infestation, and that a long shuck extension was of much value regardless of earworm entrance. Infestation by the rice weevil and stored-grain insect species was negatively correlated with the length of shuck. Cartwright also showed that earworm infestation increased the possibility and probability of infestation by the rice weevil. Eden showed that length of shuck tip and number of leaves per husk were important constituents of husk cover in the prevention of damage by the rice weevil. The effect of each factor was shown to be independent of the other, and a combination of both gave the least amount of weevil damage. Another characteristic of corn which is commonly associated with rice weevil resistance is that of kernel hardness. The harder the corn the more resistance it is supposed to possess. Eden, working with the hardness of various corn varieties in 1952, reached the conclusion that weevil damage decreased approximately 1% for each 1 pound per square inch increase in pressure required for kernel penetration.

Recent surveys conducted to determine the damage to corn in Louisiana by stored grain insects revealed the following facts. In 1955 infestation records made from the field at the time of harvest, or immediately thereafter, showed that the average kernel damage or infestation for the state was 8.67%. Thirty-seven per cent of the ears were infested. Corn from the same cribs in May of 1956, after approximately 6 to 7 months' storage on the farms averaged 13.5% kernel damage, and in August this damage had increased to 21.6%. The species responsible for most of this damage was the rice weevil. After several months the Angoumois grain moth, *Sitotroga cerealella* (Oliv.) was of some importance, especially in north Louisiana. It is of interest to note that the infestation of corn in Louisiana by the rice weevil varies to the extent that certain areas—the Ouachita Valley and upper Mississippi River Valley—are practically free of damage while other areas—south Louisiana and north Louisiana

Accepted for publication July 8, 1957.

hill country—are so heavily damaged that much of the corn when harvested is hardly worthy of storage. Corn from several fields in southwest Louisiana in 1955 was found to contain as much as 90% of the kernels damaged before harvest. Such corn is literally of no value and is termed "pig-feed" locally.

These differences in infestation in corn by the rice weevil from several ecological areas of the state cannot be attributed to temperature differences. The reason is unexplained to date. The writers suggest that it may be related to the overall effects of the wholesale insecticide program commonly employed in cotton production in the Ouchita and upper Mississippi River Valleys. An investigation of this possibility is now underway.

The heavy rice weevil infestations in corn grown in Louisiana at the time of harvest suggested the study of some possible factors other than husk length which might influence this initial infestation in the field, and the effects of various insecticidal treatments on field infestation.

PROCEDURE.—A field experiment was conducted to study the relationships between earworm infestation, bird-damaged ears, and certain insecticidal treatments applied to the developing or the mature ear. Louisiana hybrid 521 was selected for the study. This is a popular white corn having what is considered good husk coverage, but it is also susceptible to damage by the rice weevil and is nearly always severely damaged in the field in Louisiana by this species.

A 3-acre field of this corn was grown by normal planting and cultivating practices. Treatments began at first silking and continued for several weeks. A 3-gallon compressed-air sprayer was used to apply the insecticides. A pressure of 40 pounds was maintained during the spraying operation.

Treatments employed and the schedules of applications were as follows:

1. 2 pounds of DDT plus 1 gallon of white mineral oil—individual ear treatment. 5 applications at 2-day intervals beginning at first silking[2] (June 14).
2. Same spray—simulated machine application. One application made on the date of no. 5 application in treatment 1 (June 22).
3. Same spray—simulated machine application. One application made 2 weeks after treatment 2 (July 6).
4. Same spray—simulated machine application. One application made 4 weeks after treatment 2 (July 20).
5. Same spray—simulated machine application. One application made 6 weeks after treatment 2 (Aug. 3).
6. Same spray—simulated machine application. One application made 8 weeks after treatment 2 (Aug. 17).
7. Same spray—simulated machine application—two applications. Application no. 1 made at same time as treatment 3, and application no. 2 four weeks later (July 6–Aug. 3).
8. 1 pound of endrin plus 1 gallon of White mineral oil. Individual ear treatment. One application made at time of treatment 3 (July 6).
9. Same spray as in treatment 8. Individual ear treatment—two applications made at time of treatment 7 (July 6–Aug. 3).
10. Ears caged with hardware cloth cones. (Purpose: to protect the shuck from bird damage.)
11. Untreated check.
12. Two pounds of DDT plus 1 gallon of white mineral oil—simulated machine application. Three applications at 3-day intervals beginning at first silking.
13. Same spray—simulated machine application. Five applications at 3-day intervals beginning at first silking.
14. Thirty pounds of 10% DDT dust per acre. One application made at time of treatments 3 (July 6).
15. Thirty pounds of 10% DDT dust per acre—two applications. Application no. 1 made at time of treatment 3, followed in 4 weeks by application no. 2 (July 6–Aug. 3).
16. Silks clipped every other day from the top ears only (to tip of shucks). (Purpose: to prevent earworm entrance into the ear in the absence of an insecticidal residue.)
17. Silks clipped every other day from the top ears only followed by caging these ears in the dough stage with hardware cloth cones. (Purpose: earworm control in the absence of an insecticidal residue and protection of the ears from bird damage.)

The experimental design consisted of a randomized-block arrangement. Each replicate was six rows wide by 70 feet long and contained 1/29 acre. Each of the 17 treatments was replicated four times. Plants were spaced 12 inches in the row making a total of 420 plants per replicate. The field was planted March 26. The first spray application was made June 14. Subsequent applications were made as scheduled.

When the kernel moisture reached 15% (Sept. 18), the plots were harvested and records were made of the damage to the ear or shuck by birds, the earworm infestation, per cent of kernels damaged by the rice weevil, and the number of ears containing infestation by the pink corn scavenger, *Pyroderces rileyi* (Wals.). The above records were made from 100 top ears taken at random from the two centers rows of each plot. The ears were examined for the various damage categories immediately after harvest. After the examination was completed the ears were shelled and the total weight of each 100-ear sample obtained. The grain from each replicate was then thoroughly mixed and a 1-quart sample removed and treated with 2 p.p.m. of lindane[3] to permit continued development of the rice weevil larvae but prevent further oviposition by newly emerged adults (Floyd & Newsom 1956). These samples were stored in cardboard cartons at room temperature for 5 weeks, after which they were opened, remixed and the kernel damage by the rice weevil determined by individually examining 1000 kernels in lots of 100 each from each replicate. After this examination was completed the weights per bushel were determined (table 1).

Twenty-pound samples from all replicates of several of the treatments were placed in cloth bags, tied, and suspended from the rafters of a storage crib. After 7 months' storage and exposure to rice weevils these samples were examined as described above for rice weevil damage (table 2).

RESULTS AND DISCUSSION.—Table 1 shows the relative values of the several treatments in protection of the corn in the field from damage by the rice weevil. It also shows that infestation of corn in the field by the rice

[2] The DDT was obtained from a commercial grade of emulsifiable concentrate. The amount of chemicals given represents the amounts applied per acre. The total amount of liquid used was 25 gal. per acre.

[3] Technical lindane in solution absorbed on dry sawdust and scattered uniformly throughout the grain mass.

Table 1.—Effects of several treatments on rice weevil damage to corn at time of harvest. Baton Rouge, La. 1956.

Treatment No.	Per Cent of Ears Damaged by—			Per Cent of		Weight of 100 Shelled Ears (Pounds)	Weight per Bushel (Pounds)	Grain Moisture (Per Cent)
	Birds	Earworms	Rice Weevils	Kernel Damage by Rice Weevils	Ears Infested by Pink Corn Scavenger			
1	8.70	17.7	8.0	2.0	79	44.5	58.4	13.15
2	9.20	54.5	19.2	2.7	72	45.8	57.7	13.26
3	11.70	58.7	24.7	5.3	99	43.4	56.7	13.08
4	12.70	61.0	8.0	1.8	97	43.5	58.2	12.91
5	13.70	59.7	25.0	5.5	98	45.4	57.1	12.71
6	11.70	61.0	16.7	4.5	97	43.0	57.2	13.20
7	13.20	62.5	16.0	4.6	94	43.5	57.3	12.98
8	8.20	54.5	4.5	0.8	39	45.7	58.5	13.22
9	11.20	51.7	2.5	0.2	14	46.6	58.9	13.35
10	0.25	48.5	8.7	1.5	97	46.0	57.6	13.24
11	13.20	62.2	26.2	4.1	97	43.4	57.2	13.08
12	12.00	49.7	41.0	8.6	98	43.5	56.7	12.81
13	9.70	41.2	15.5	2.0	92	44.0	57.5	12.85
14	12.20	57.2	22.5	4.7	98	42.8	57.4	13.16
15	9.70	60.7	32.0	5.8	94	42.3	57.0	12.93
16	11.50	38.5	26.0	5.3	94	43.8	57.2	13.00
17	0.50	23.0	5.0	0.9	95	45.0	58.0	13.32
L.S.D. at 5%	6.2	11.1	16.1	3.8	13	3.3	0.9	
at 1%	8.3	14.9	21.5	5.0	17	4.2	1.2	

weevil is positively related to bird damage and earworm infestation.

The oil-DDT sprays applied 3 or 5 times resulted in a significant reduction in the earworm infestation. However, only five applications applied directly to the silks (treatment 1) resulted in a practical reduction of the earworm infestation. This same schedule and method of application also reduced, though not significantly, damage by the rice weevil. The endrin sprays, treatments 8 and 9, were applied too late to have any effect on the earworm infestation. However, both treatments greatly influenced rice weevil damage. Two applications of endrin (treatment 9) reduced infestation by the rice weevil significantly to 0.2% kernel damage. The single application (treatment 8) reduced the infestation to 0.8% damage. These same treatments, 8 and 9, significantly reduced infestation by the pink corn scavenger caterpillar, a pest that is very abundant and highly destructive to corn in Louisiana. The difference obtained in rice weevil control between the 1 and 2 applications of endrin cannot be postively identified with the numbers of applications made. The difference could have been influenced by a peak appearance of the rice weevil in the field (table 3).

Another test conducted in a nearby field with a hybrid highly susceptible to the rice weevil (Funks G-134) designed to determine the earliest appearance of the various stored grain insect species in the field had shown that the first emergence of adults from field oviposited eggs occurred July 26 and continued thereafter for several days. In view of this, it is possible and very probable that the second application in the 2-application treatment of endrin was of more importance in rice weevil control than the first. The importance of timing is shown also in treatment number 4. The rice weevil damage did not

Table 2.—A comparison of the effects of several treatments on rice weevil damage to corn at harvest time and after 7 months' storage. Baton Rouge, La. 1956.

Treatment	Per Cent Rice Weevil Damage		Weight per Bushel		Grain Moisture		Insecticide Residue[a] (p.p.m.)		
	At Harvest	After 7 Mo.	At Harvest	After 7 Mo.	At Harvest	After 7 Mo.	Grain	Ear Zone	Stalk
No. 1—(5 DDT sprays at 2-day intervals)	2.0	68	58.4	52.0	13.15	12.28	0	56.2	85.2
No. 8—(1 endrin spray)	0.8	45	58.5	54.2	13.22	12.41	0	0.6	1.7
No. 9—(2 endrin sprays)	0.2	14	58.9	56.7	13.55	12.30	0	4.6	0.5
No. 10—(Wire caged ears)	1.5	77	57.6	50.6	13.24	11.92	—	—	—
No. 11—(Check)	4.1	82	57.2	49.2	13.08	12.28	0	0.0	0
No. 12—(3 DDT sprays at 3-day intervals	8.6	90	56.7	45.0	12.81	12.63	0.2	4.4	8.5
No. 16—(Silks clipped)	5.3	86	57.2	47.9	13.00	12.73	—	—	—
No. 17—(Silks clipped and ears caged)	0.9	56	58.0	53.0	13.32	12.16	—	—	—

[a] Residue analysis made by the Pesticide Chemical Research Laboratories, U. S. Dept. of Agriculture.

Table 3.—Relative abundance of the rice weevil in the field in 1956.[a]

DATE	AVERAGE NUMBER OF ADULTS PER EAR	PER CENT EARS INFESTED	KERNEL MOISTURE (PER CENT)
6-14	0	0	84.2
6-18	0	0	77.0
6-21	0	0	79.0
6-25	0	0	64.5
6-28	0.26	13	60.0
7-2	0.28	17	52.0
7-5	0.42	27	43.0
7-9	1.20	45	37.5
7-13	2.19	66	35.0
7-16	3.73	83	33.0
7-19	3.87	81	30.0
7-23	2.25	74	27.0
7-26	5.40	86	25.0
7-30	—	75	25.0
8-10	6	92	19.5
8-16	7.58	98	16.7
8-23	14.65	95	14.0

[a] These figures obtained from a field of Funks G-134 growing 200 yards from the test field of La. 521.

differ significantly from the other treatments; however, it is quite low in comparison with most of the other treatments. This treatment (one application of DDT spray) was made July 20, the approximate date when the peak appearance and emergence of adult rice weevils occurred (table 3).

The difference in rice weevil infestation between treatments 12 and 13 may have been due to the lower number of early applications of DDT in treatment 12 which resulted in an insufficient residue to kill the entering weevils, but was possibly sufficient to adversely affect some unknown parasite or predator of the weevil. Treatment 13 received five applications which extended the schedule 1 week longer in addition to building up a greater residue which possibly could have been adequate to kill some of the entering weevils later. Results from treatments 10, 16, and 17 designed to evaluate the importance of earworm infestation and bird damage to rice weevil infestation show that those ears that had protection from bird damage only (treatment 10) contained approximately one-third as much rice weevil damage as the untreated control. When the ears were protected from both earworms (by clipping the silks) and bird damage the kernels contained only 22% of the rice weevil damage as compared with the control. Where the silks were clipped (treatment 16) to effect earworm control in the absence of insecticidal residue, but with the ears left exposed to bird damage, the rice weevil infestation was not affected. However, in this treatment the earworm infestation was not reduced to the extent it was in treatment 17. Compared with the infestation in the check—4.1% kernel damage—treatment 17 resulted in 0.9% damage, the normal infestation where, theoretically, neither birds nor earworms were factors. Assuming then that 0.9 represents the infestation as determined by causes due to inherent characters of the corn, and 4.1% as the infestation figure as a result of these inherent characteristics plus physical disturbances of the shuck coverage (earworm and birds), 22% of the actual damage resulted from the inherent factors and 78% as a result of shuck disturbance as influenced by earworms and birds. When the damage attributed to inherent factors, 0.9%, is taken from the damage attributed to both inherent factors and earworms, 1.5%, a value of 0.6% kernel damage or 15% of the total damage can be attributed to the earworm as the predisposing factor. Where the bird was the only shuck disturbing factor[4] (treatment 16) the rice weevil damage was equal to the control. Therefore, 63% of the rice weevil damage can be attributed to birds as the predisposing factor.

SUMMARY.—Results from examinations made of corn from the various ecological areas in Louisiana revealed that damage to corn at harvest averaged more than 8% kernel damage due largely to the rice weevil, *Sitophilus oryza* (L.). The progressive loss in storage increased until at the end of 10 months the kernel damage averaged 22 per cent. The degree of damage varied considerably. Generally the north Louisiana hill country, and south and southwest Louisiana, and southeast Louisiana suffered the greatest damage. The Ouachita Valley was found to be almost free of damage. The upper Mississippi and Red River Valleys were relatively free of damage.

A field experiment was conducted which was designed to study the effects of factors other than husk coverage which might influence field infestation by the rice weevil. Corn earworm infestation, the shuck condition as influenced by birds, and the effects of several insecticides applied at various timing schedules were studied. DDT dusts and sprays failed to give a significant reduction in rice weevil damage; however, two sprays of endrin applied to the ears on July 6 and August 3 resulted in almost complete control of the rice weevil.

The main pre-disposing factor which influenced rice weevil infestation in the field in this study was bird damage to the husk. Corn earworm damage alone was responsible for approximately 15% of the total damage, whereas the birds were responsible for 63%. The remainder of the damage was attributed to normal inherent characteristics of the corn.

REFERENCES CITED

Back, A. E. 1919. Conserving corn from weevils in the Gulf States. U. S. Dept. Agric. Farmers' Bull. **1029.**

Cartwright, O. L. 1930. The rice weevil and associated insects in relation to shuck lengths and corn varieties. South Carolina Agric. Expt. Sta. Bull. **226.**

Eden, W. G. 1952. Effect of husk cover of corn on rice weevil damage in Alabama. Jour. Econ. Ent. **45:** 543–4.

Eden, W. G. 1952. Effects of kernel characteristics and components of husk cover on rice weevil damage to corn. Jour. Econ. Ent. **45:** 1084–5.

Floyd, E. H., and L. D. Newsom. 1956. Protection of stored corn with lindane-impregnated sawdust. Jour. Econ. Ent. **49:** 753–7.

Kyle, C. H. 1918. Shuck protection for ear corn. U. S. Dept. Agric. Professional Paper Bull. **708.**

Massop, M. C. 1940. Control of maize weevil, *Calandra oryzae* L. Rhodesia Agric. Jour. **37:** 461–75; 517–28.

[4] Actually, the earworm control obtained in this treatment (No. 16) was only a little greater than that obtained in treatment No. 17.

Types and Seasonal Incidence of Plant Bug Injury to Peaches[1]

ROY W. RINGS, *Ohio Agricultural Experiment Station, Wooster*

For the past 4 years, 1953–56, investigations have been carried on to determine the insect species responsible for producing cat-facing injury and related types of damage to peaches. The results of this work are reported in a series of publications of which this paper forms a part. The results of cage experiments with stink bugs were reported by the author (1957) and the phases dealing with the plum curculio, the meadow spittlebug and other miscellaneous species will appear at a later date. The present paper is concerned only with the types of injury produced by various species of plant bugs and their seasonal occurrence.

Apparently the first publication referring to peach injury by plant bugs was the report of Lowe (1900). He states that the "tarnished plant bug" was responsible for producing cat-facing and gummosis in a peach orchard near Rochester, New York. However, since the plant bugs he describes were numerous from June 11 to June 30, a period during which tarnished plant bugs are not normally abundant in orchards, it is suspected that his identification was incorrect. Furthermore, most of the injury occurred on trees bordering a woods containing chestnut, white oak and sassafras and it is quite probable that this injury was caused by one of the oak plant bugs, which were not described by Knight until 1917.

Plant bug injury was next described by Taylor (1908) in Missouri and the species involved was undoubtedly the tarnished plant bug, *Lygus lineolarius* (P. de B.). A great abundance of tarnished plant bugs was noted feeding on the buds and newly opened blossoms of early blooming peach varieties. Many of the blossoms showed a darkening and shrinking and some were dropping as a result of plant bug injury.

The first authentic record of oak plant bug injury to peaches was that of Caesar (1921) who reported severe injury by the white oak plant bug, *Neolygus* (*Lygus*) *quercalbae* Knight, at St. Davids in the Niagara peninsula of Ontario. The damaged fruit was badly scarred but only a small portion of the affected fruit was knobby. This report mentions that Professor P. J. Parrott of the New York Agricultural Experiment Station also found *Neolygus quercalbae* and the hickory plant bug, *Neolygus* (*Lygus*) *caryae* Knight, feeding on peaches in New York State during the same year. Ross (1922) mentioned damage to peaches by *Neolygus quercalbae* and the oak plant bug, *Neolygus* (*Lygus*) *omnivagus* Knight, in 14 orchards near St. Catharines, Ontario. In one orchard 75% of the fruit had been injured. In 1923 Thatcher stated that *Neolygus caryae* and *Neolygus quercalbae* were responsible for serious fruit deformation in peach orchards adjacent to woodlands in New York State.

The first detailed study of tarnished plant bug injury to peaches was made by Porter, Chandler and Sazama, who reported their results in 1926 and 1928. These investigations proved that the tarnished plant bug was not only responsible for a considerable amount of blossom and fruit drop but also that it was associated with "cat-faced" or deformed peaches which were quite prevalent in orchards located in southern Indiana and southern Illinois. Rice (1937) in Delaware, Woodside (1946) in Virginia, and Rings (1956) in Ohio, found that similar tarnished plant bug injury occurred in their respective areas. Injury to peaches by *Lygus lineolarius*, *Neolygus caryae*, *N. omnivagus* and *N. quercalbae* has also been described by Garman (1928) in Connecticut.

METHODS.—Cage tests were conducted with the tarnished plant bug from 1953 to 1956 to determine the various types of injury produced and the relationship between the type of injury and the seasonal development of the fruit. Exploratory studies were begun in 1955 to determine whether or not other species of plant bugs were involved in Ohio. The selection of species for study in 1956 was based upon their performance in cage tests in 1955. In 1956 several species of *Neolygus* as well as other species were used in cage tests during the period when the adult insects were active. The cages used, the observations on feeding habits and the type of injury produced were the same as those described by the author in a previous paper (Rings 1957).

Tarnished plant bugs were collected as needed by sweeping clover or alfalfa fields. Oak and hickory plant bugs, as well as other species, were collected by jarring various species of hickories, oaks and other trees over an inverted umbrella lined with white nylon.

In order to correlate insect injury with the seasonal development of the fruit, Elberta peaches were sampled each week and their diameter and volume were measured by methods previously described.

SPECIES OF PLANT BUGS PRODUCING INJURY IN PEACHES.—A list of the species of Miridae which were employed in cage tests and the types of injury produced are shown in table 1. All species were initially identified by R. I. Sailer of the Section of Insect Identification and Parasite Introduction, Entomology Research Division, Agricultural Research Service. It may be noted that *Lygus lineolarius* as well as several specimens of *Neolygus* produced several types of injury. Only four of the other eight species tested produced visible injury to exposed peaches.

TYPES OF INJURY PRODUCED BY PLANT BUGS.—Although there was some variation and some intergradation in the injuries produced by various species of plant bugs, these injuries could be classified into several general categories.

Blossom or fruit drop.—Blossoms which were attacked by tarnished plant bugs withered and dropped. Also a considerable portion of the fruit attacked by this species from petal-fall to shuck-fall dropped as a result of the feeding injury. In 1955 approximately 20% of the dropping was due to plant bug injury, while in 1954 about

[1] This project was supported cooperatively by the Ohio Agricultural Experiment Station and the Section of Fruit Insect Investigations, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture. Accepted for publication July 12, 1957.

Table 1.—Importance of plant bugs and types of injury produced in peaches at different stages of development.

Species	Economic Importance	Number of Cage Tests	Seasonal Incidence of Injury Expressed in Number of Days After Bloom				
			Cat-faced and Gummy	Scarred and Gummy	Water-soaked	Gummy Only	Blossom or Fruit Drop
Ceratocapsus nigellus Knight	None	9			63	63	
Deraeocoris quercicola Knight	None	10					
Leptoterna dolabratus (L.)	None	12				42	
Lygus lineolarius (P. de B.)	Important	125	14–35	35–42	35–70	49–91	0–30
Neolygus canadensis Knight	None	5	35			56	
Neolygus caryae Knight	Important locally	29	35	35–42	42–49	42–49	
Neolygus geneseensis Knight	None	5			56	49–70	
Neolygus neglectus Knight	None	1		35			
Neolygus omnivagus Knight	Important locally	39	28–35	35–42	42–56	49–70	
Neolygus quercalbae Knight	Important locally	23	35	35		42–49	
Paracalocoris scrupeus (Say)	None	4					
Phytocoris infuscatus Reuter	None	6				56	
Phytocoris puella Reuter	None	4					
Phytocoris salicis Knight	None	4				56	
Stenotus binotatus (Fab.)	None	4					

37% of the drop was attributed to injury by this species. Blossom drop is not caused by the various species of *Neolygus* since they do not appear in the orchard until the fruit is beyond the shuck-fall stage.

Cat-facing injury.—This type of damage included all types of injury involving fruit deformation or distortion at harvest. Cat-facing, which is shown in figure 1, A, resulted from plant bug feeding during the period from shuck-split until the fruits reached a diameter of about 20 millimeters. Usually this damage consisted of shallow to deep depressions, which were covered with a brown, corky tissue. The scars were devoid of pubescence except for small, scattered patches of grayish or brownish fuzz. There was usually a mass of gum centrally located in the affected area. Scars varied in width from 2 to 26 millimeters and the larger ones undoubtedly represented the coalescence of several smaller scars. Some fruits which were about 20 millimeters in diameter when injured developed deformities without scarring or fuzz removal. Peaches that were attacked earlier usually dropped, while those larger than 20 millimeters in diameter showed scarring but normally no fruit deformation at harvest.

Scarred injury.—This type of damage was more frequently encountered with plant bugs than with stink bugs. Typical injury consisted of a group of brownish scars concentrated in a relatively small area as shown in figure 1, B. The brown, corky, scarred areas were devoid of pubescence except for small patches of discolored fuzz and a small mass of gum was usually found in the median area. The width of the scars varied from 1 to 2 millimeters but in some cases many of these coalesced to form larger scars, some of which measured 9 by 25 millimeters. There was a concentration of reddish pigment in the skin around the edges of some of the scars.

Water-soaked injury.—Fruit showing this type of injury (fig. 1, C) had small dark-green areas which had a water-soaked appearance. These were not depressed as was the case in some stink bug injury. Water-soaked areas were roundish in shape and in size ranged from 2 to 3 millimeters in diameter. When the skin of these fruits was removed, dark-green, water-soaked areas in the flesh could be observed. These areas were of the same size and shape as the areas on the skin and extended about 2 millimeters deep. This type of injury caused by plant bugs was not associated with gummosis although both types of injury often occurred on the same fruit.

Gummosis injury.—This condition was characterized by an exudation of clear gum after the epidermal tissues were punctured by feeding insects. Gummosis was quite often associated with cat-facing and scarring but this response also occurred alone. Early in the season injured fruits exuded gum in long strings but this was usually followed by cat-facing or scarring unless the fruit dropped. Later in the season when gummosis only was produced the gum was exuded in small droplets (fig. 1, D). Occasionally the gummy exudate would be darkened by the growth of a sooty fungus.

There was only a small amount of flesh injury associated with plant bug gummosis as compared with that produced by stink bugs. The discolored area, beneath the skin, was roundish in shape and about 2 millimeters wide. These areas extended only from 2 to 3 millimeters beneath the skin whereas in the case of stink bug injury they extended from 5 to 16 millimeters deep.

SEASONAL INCIDENCE OF THE VARIOUS TYPES OF INJURY.—An attempt was made to correlate the seasonal incidence of various types of injury with the seasonal development of the peach fruit. A graphic correlation of this relationship is shown in figure 2. The diameter of the fruit in millimeters and the volume of the fruit in milliliters were plotted against the number of days after bloom. A cyclic growth of the fruit may be noted from the growth curves in figure 2. This development may be divided into three stages. In Stage I the fruit increases rapidly in size. Stage II is characterized by a reduced rate of growth which is coincident with the hardening of the kernel and the development of the endoderm and embryo to approximately maximum size. In Stage III the rate of growth again increased rapidly, largely as a result of the enlargement of cells in the flesh.

The seasonal incidence of the various types of injury produced by plant bugs was somewhat different from

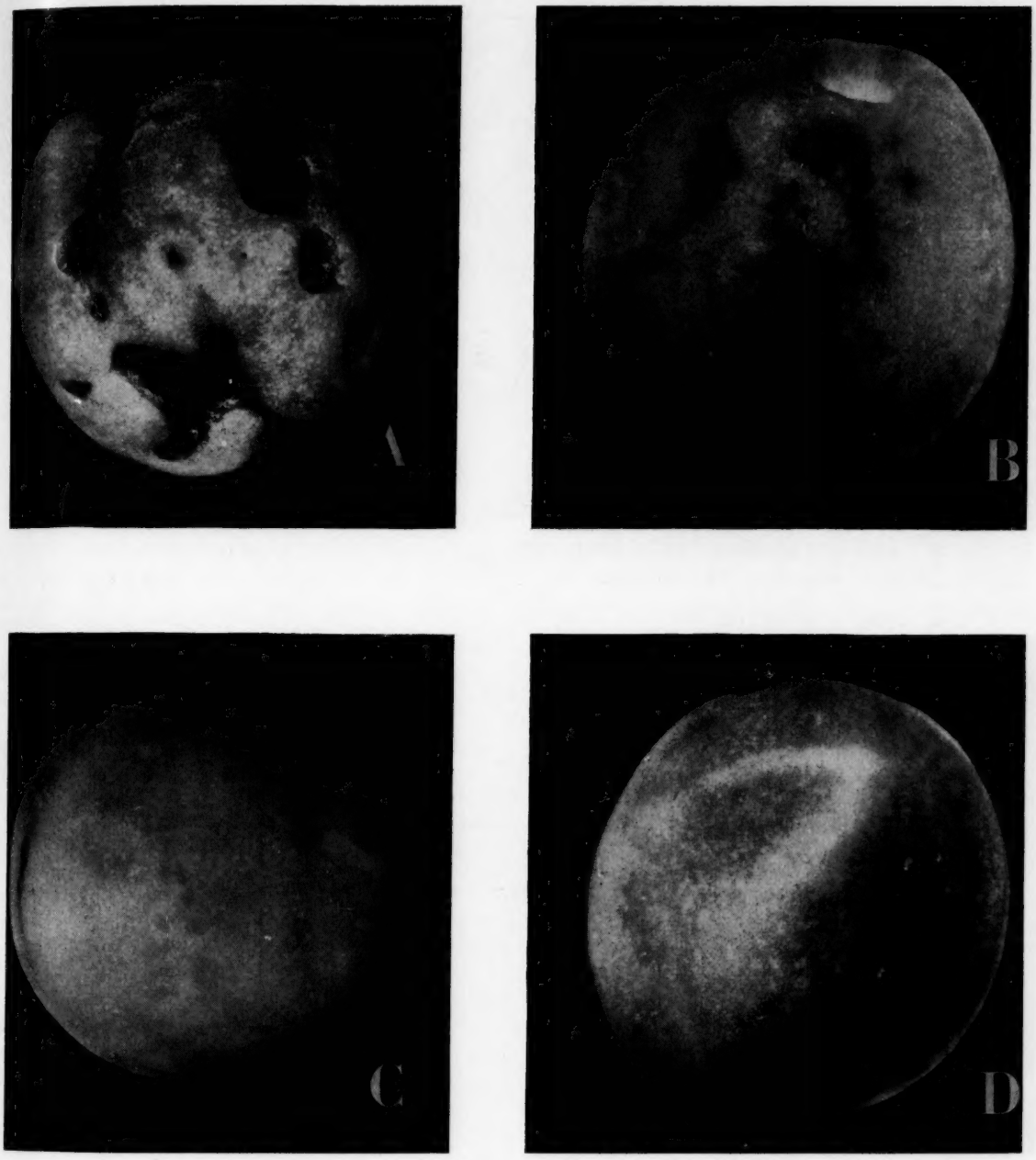


FIG. 1.—Elberta peaches showing various types of injury produced by plant bugs.

A. Cat-facing injury produced by the tarnished plant bug, *Lygus lineolarius*
B. Scarring and gumming injury produced by the hickory plant bug, *Neolygus caryae*.
C. Water-soaked injury produced by the white oak plant bug, *Neolygus quercalbae*.
D. Gummosis injury produced by the oak plant bug, *Neolygus omnivagus*.

that produced by stink bugs. In general, cat-facing was produced later in the season than stink bug cat-facing. Scarring occurred much more frequently as a result of plant bug feeding than as a result of stink bug feeding. Gummosis was not produced as late in the season by plant bugs as by stink bugs.

The tarnished plant bug.—This species caused dropping of blossoms and fruit from bloom to about 30 days after bloom by piercing and injuring these parts in feeding activities. Cat-facing was produced from the shuck-split stage (14 days after bloom) to 35 days after bloom (fig. 2). Most of the fruit attacked in the shuck-split stage dropped as a result of plant bug attack. The peak of cat-facing injury occurred within a few days after shuck-fall. Since tarnished plant bugs are quite abundant at peach bloom and their numbers gradually decrease as the season pro-

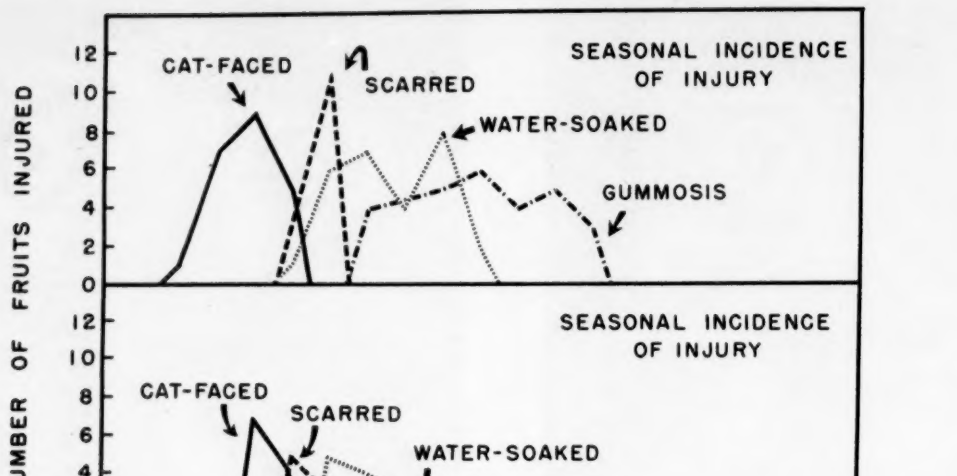


FIG. 2.—Graphic correlation between seasonal incidence of various types of injury produced by *Lygus lineolarius* (upper graph), *Neolygus omnivagus* (middle graph), and the seasonal development of the fruit (lower graph).

gresses, dropping and cat-facing are the most important types of injury produced by this species. Cat-facing injury was closely followed by scarring and gummosis injury which occurred from 35 to 42 days after bloom. Water-soaked injury occurred frequently from 35 to 70 days after bloom. Gummosis was often associated with cat-facing and scarring but not with water-soaked injury. Some fruits, however, did show both water-soaked and gummosis injury, particularly those attacked between 49 and 70 days after bloom. Gummosis alone was frequently encountered from 49 to 91 days after bloom.

The oak plant bug (*Neolygus omnivagus* Knight).—This species overwinters in the egg stage and the eggs hatch at about the time the oak leaves first appear. In Ohio, nymphs and adults have been collected on northern red oak, *Quercus rubra* L., black oak, *Quercus velutina* Lam., and white oak, *Quercus alba* L. The nymphs feed upon the oak hosts and mature about the first of June. The adults gradually migrate from the oaks to peach trees and various other food plants. Adults were usually found in peach orchards, particularly on trees near oaks, from 28 to 70 days after bloom. The greatest numbers are found from 35 to 42 days after bloom and after this period the numbers decrease rapidly. Cage tests with the tarnished plant bug have shown that peaches are susceptible to cat-facing injury from shuck-split to 35 days after bloom. Since *Neolygus omnivagus* does not appear in the orchard until 28 days after bloom, a relatively small number of peaches are cat-faced and the deformity produced is much less severe than that produced by the tarnished plant bug (fig. 2). The most easily recognized injury produced by this species is scarring and gumming which occurs from 35 to 42 days after bloom. Water-soaked injury was produced from 42 to 56 days after bloom while gummosis alone occurred from 49 to 70 days after bloom. The various types of injury produced by the oak plant bug were similar to, and indistinguishable from, that caused by the tarnished plant bug.

The hickory plant bug (*Neolygus caryae* Knight).—This species has a life history similar to that of the oak plant bug. The eggs, however, are deposited on various species of hickory. In Ohio, nymphs and adults have been collected from shagbark hickory, *Carya ovata* (Mill.) K. Koch, and pignut hickory, *Carya glabra* (Mill.) Sweet. Although

there were a few hickories near the experimental orchards, this species was not collected from peach in 1955 or 1956. From a study of the seasonal abundance of *N. caryae* on hickory it is probable that this species migrates to peach orchards, in areas where it is abundant, at about the same time as *N. omnivagus*. The seasonal incidence of the various types of injury produced by this species is similar to that of the oak plant bug and for this reason it has not been shown in graphic form. Two varieties of this species occur in Ohio, *N. caryae caryae* and *N. caryae subfuscus*, and both subspecies produced the same types of injury. The cage tests indicated that peaches were acceptable only for a limited period. Bugs fed readily on peaches from 35 to 49 days after bloom but beyond this period none of the bugs produced injury and died within a day or two after caging. Slight cat-facing was produced when the fruit was injured 35 days after bloom. The typical scarring and gumming (fig. 1, B) occurred from 35 to 42 days after bloom. Water-soaked injury and gummosis occurred concurrently from 42 to 49 days after bloom. The injuries produced by this species could not be distinguished from those produced by the tarnished plant bug and the oak plant bug.

The white oak plant bug (*Neolygus quercalbae* Knight).—This species has been reported breeding only on white oak (Knight 1917), but adults have also been collected on red and black oak in Ohio. The occurrence of this insect on these latter species may be only a matter of the migration of adults from white oaks to other trees. This plant bug appeared in the experimental orchard 35 days after bloom and was present for a period of about 2 weeks. The types and seasonal incidence of various kinds of injury closely resembled those of the oak and hickory plant bug and no differentiating characteristics could be found. This species was much less abundant in 1956 than in 1955. The seasonal incidence of injury is shown in table 1.

The Genesee plant bug (*Neolygus geneseensis* Knight).—This species occurs on white oak along with *N. omnivagus* and *N. quercalbae* although it is much less abundant. Its life history is apparently similar to that of these two species since adults were first collected about 3 weeks after *N. omnivagus* adults appeared in the orchard. Because of its late appearance this species produced only gummosis injury which occurred from 49 to 70 days after bloom and water-soaked injury which occurred 56 days after bloom.

Other species.—The plant bug, *Neolygus canadensis* Knight, cat-faced peaches at about 35 days after bloom and produced gummosis about 56 days after bloom. This species was collected on white oak but was not abundant. In Ohio, it has not been observed to attack peaches under natural conditions but it may account for some injury in orchards which are planted near white oaks.

The plant bug, *Neolygus neglectus* Knight, produced scarring and gumming about 35 days after bloom in one cage test. The only specimen used experimentally was collected on white oak in association with other species of *Neolygus*.

Only four of the other eight species tested produced visible injury to exposed peaches. Slight gummosis was produced by *Leptoterna dolabratus* (L.), *Phytocoris infuscatus* Reuter, *Phytocoris salicis* Knight and *Ceratocapsis nigellus* Knight.

RELATIVE IMPORTANCE OF VARIOUS SPECIES OF PLANT BUGS.—The tarnished plant bug is undoubtedly the species of greatest economic importance in Ohio due to its widespread distribution. Individual orchards, however, which are near oaks and hickories may be more severely damaged by one or more of the *Neolygus* group of plant bugs than by the tarnished plant bug. The injury most commonly observed under conditions of natural infestation is that of scarring and gumming. The other species listed in table 1 either produced no injury or occur so infrequently on peaches that they are not considered of economic importance.

SUMMARY.—Cage tests were conducted from 1953 to 1956 with 15 species of plant bugs to determine the types of injury produced in peaches. Plant bugs were allowed to feed, in cages, on peaches in various stages of development from bloom until harvest. The different types of injury produced were: (1) Blossom and fruit drop, (2) Cat-facing or deformation injury, (3) Scarred injury, (4) Water-soaked injury and (5) Gummosis injury. Three species, *Lygus lineolarius* (P. de B.), *Neolygus omnivagus* Knight, and *Neolygus caryae* Knight, produced all five types of injury. No blossom drop and very little fruit drop resulted from the feeding of these two species of *Neolygus*. *Neolygus quercalbae* Knight produced cat-faced, scarred and gummosis injury. *Neolygus canadensis* Knight produced cat-facing and gummosis while *N. neglectus* caused only scarring and gumming. Slight gummosis was produced by *Neolygus geneseensis* Knight, *Leptoterna dolabratus* (L.), *Phytocoris infuscatus* Reuter, *Phytocoris salacis* Knight and *Ceratocapsis nigellus* Knight.

Peaches in different stages of growth responded in different ways to plant bug feeding injury. Blossoms which were attacked by the tarnished plant bug withered and dropped. A considerable portion of the fruit attacked by this species from petal-fall to shuck-fall dropped as a result of tarnished plant bug feeding. Cat-facing injury was produced from 14 to 35 days after bloom by *Lygus lineolarius* and various species of *Neolygus*. These species also produced scarring and gumming from 35 to 42 days after bloom. Water-soaked injury was produced by several species from 35 to 70 days after bloom. Gummosis was associated with most of the previously mentioned types of injury but also occurred alone from 42 to 70 days after bloom.

REFERENCES CITED

Caesar, L. 1921. Notes on leaf bugs (Miridae) attacking fruit trees in Ontario. Ent. Soc. Ontario, Ann. Rept. for **1920**: 14–6.

Garman, Philip. 1928. Plant bug injury to fruits. Connecticut Agric. Expt. Sta. 28th Rept. of State Entomologist Bull. **305**: 729–31.

Knight, Harry H. 1917. A revision of the genus *Lygus* as it occurs in America north of Mexico, with biological data on the species from New York. New York Agric. Expt. Sta. (Cornell) Bull. **391**: 643–737.

Lowe, V. H. 1900. Miscellaneous notes on injurious insects. New York Agric Expt. Sta. (Geneva) Bull. **180**: 135.

Porter, B. A. 1926. The tarnished plant bug as a peach fruit pest. Jour. Econ. Ent. **19**: 43–8.

Porter, B. A., S. C. Chandler and R. F. Sazama. 1928. Some causes of cat-facing in peaches. Illinois Nat. History Survey Bull. **17**(Article 6): 261–75.

Rice, Paul L. 1937. Cat-facing of peaches by the tarnished

plant bug, *Lygus pratensis* (L.). Trans. Peninsula Hort. Soc., pp. 1–6.

Rings, Roy W. 1956. Insect and mite pests of peaches in Ohio. Ohio Agric. Expt. Sta. Res. Bull. 768: 1–48.

Rings, Roy W. 1957. Types and seasonal incidence of stink bug injury to peaches. Jour. Econ. Ent. 50(5): 599–604.

Ross, W. A. 1922. Insects of the season in Ontario. Ent. Soc. Ontario, Ann. Rept. for 1921: 42–52.

Taylor, Estes P. 1908. Dimples in apples from oviposition of *Lygus pratensis* L. Jour. Econ. Ent. 1: 370–5.

Thatcher, R. W. 1923. Peach deforming capsids. New York Agric. Expt. Sta. (Geneva) 41st Ann. Rept., p. 39.

Woodside, A. M. 1946. Some insects that cause cat-facing and dimpling of peaches in Virginia. Virginia Agric. Expt. Sta. Bull. 389: 1–15.

Seasonal Occurrence of Fleas on Antelope Ground Squirrels in the Great Salt Lake Desert[1]

DALE D. PARKER, *Ecological Research, University of Utah, Dugway*

The antelope ground squirrel (*Citellus leucurus leucurus* (Merriam)) population and its associated flea fauna have been known to harbor *Pasteurella pestis* in nature (Pollitzer 1954). In the Great Salt Lake Desert area, fleas from this squirrel were found to have been naturally infected with *Pasteurella tularensis* (Woodbury & Parker 1954). The implications that hosts and fleas are associated with these diseases suggest the possibility of transmission to humans or to livestock. Further investigation into the seasonal population dynamics of the fleas on antelope ground squirrels was initiated in order to obtain a better understanding of the ecology of the host and ectoparasite species as related to the epizoology of the diseases.

Seasonal fluctuations of the flea fauna on the California ground squirrel, *Citellus beecheyi* (Richardson) in a semi-desert climate have been reported by Stewart and Evans (1940), Linsdale (1946) and Holdenried *et al.* (1951). The contributions made by these workers provided basic reference sources upon which survey or control programs in coastal regions might be based, and information upon which this investigation was established.

Antelope ground squirrels range throughout desert regions west of the Rocky Mountains from southern Baja California north to east central Oregon. The subspecies, *Citellus leucurus leucurus* (Merriam), studied in this investigation, occurs over a major portion of the range of the entire species (Miller & Kellogg, 1955). This investigation is confined to a single locality in the eastern edge of the range of this subspecies, in which trapping of squirrels was conducted during a period extending from March 1955 to March 1956.

The author wishes to acknowledge assistance given by members of the Ecological Research staff of the University of Utah for making collections during parts of the year. J. Franklin Howell and Carlo M. Ignoffo provided technical assistance.

DESCRIPTION OF AREA.—Squirrels were collected from a juniper-brush community extending in a finger-like projection onto the flats from the foothills of the Cedar Mountains, Tooele County, Utah. In the immediate trapping area the native juniper (*Juniperus utahensis* (Engelm.) Lemmon, is interspersed with the desert plants: four-wing saltbush, *Atriplex canescens* (Pursh.) Nutt.; rabbitbrush, *Chrysothamnus* spp., horsebrush, *Tetradymia glabrata* A. Gray; greasewood, *Sarcobatus vermiculatus* (Hook) Torr. and Indian ricegrass, *Oryzopsis hymenoides* (R. and S.) Richer. Much of the surrounding area is covered by both stabilized and unstabilized sand dunes. Antelope ground squirrels are active in the daytime along the edges of the dunes and in the vegetated areas between the dunes (see fig. 1).

METHODS—Trapping within the juniper-brush community was confined to approximately five acres in which soil types and vegetation were similar. Five to fifteen squirrels were trapped and examined every two weeks. Previous experience indicated that this number could be taken periodically without entirely depleting the population before completion of the project.

Trapped animals were placed alive in individual flea-proof cloth bags and transported to the laboratory for processing. The animals were killed and their carcasses brushed over a white enameled pan. After having been stored in a refrigerator at 4° C. for at least 24 hours, they were brushed a second time. The second brushing often yielded additional fleas presumed to have been attached at the time of the initial brushing. The remaining fleas were more easily removed if carcasses were allowed to thaw slightly before they were brushed. Ectoparasites recovered from the pan were stored in 70% alcohol before being cleared, permanently mounted and identified.

The index for a given flea species was determined for a given period by dividing the number collected by the number of squirrels examined. In this study all indices have been projected for either a monthly or yearly period.

Daily records of temperature and relative humidity were kept by the Dugway weather station for an area approximately five miles west of the study plot and 100–200 feet lower in elevation.

RESULTS.—During the 12-month investigation 2,715 fleas were collected from 138 squirrels. The most abundant species, *Thrassis bacchi gladiolis* (Jordan) was collected during every month of the year. This species outnumbered all other species collected, except during July, August and September, when a decrement in the species index was observed. During these months mean temperature increased and relative humidity decreased markedly. The index for *T. b. gladiolis* reached a minimum (3.4) during September, but as the mean temperature decreased to below 64° F. and relative humidity increased to above 40

[1] This project was supported by U. S. Army Chemical Corps contract, No. DA-18-064-CML-2639, with the University of Utah. Accepted for publication July 18, 1957.

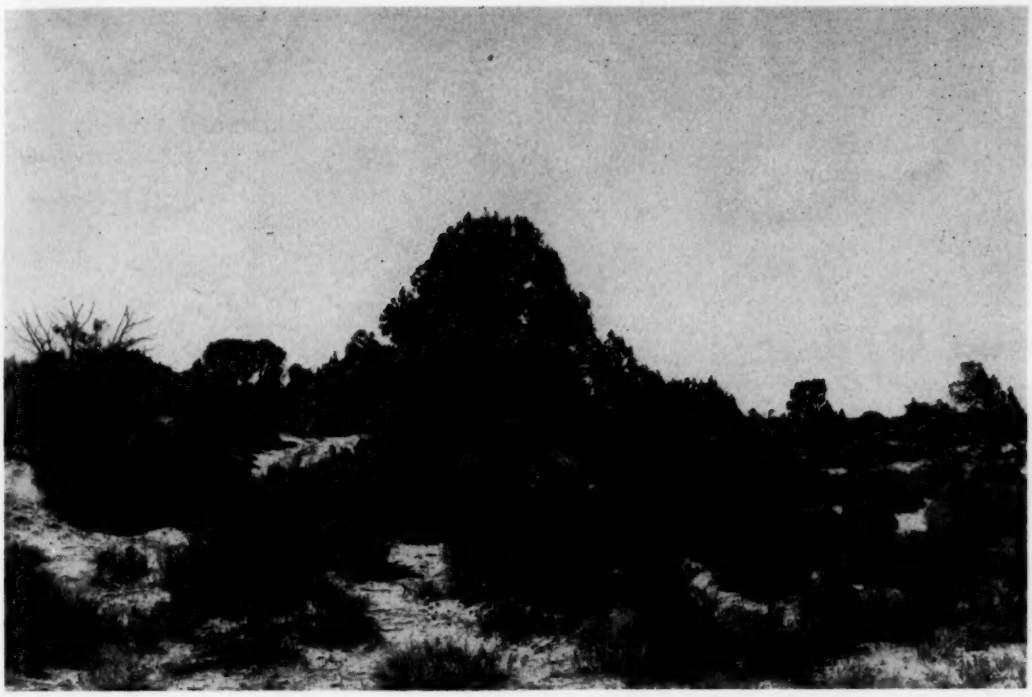


FIG. 1.—View of study area in typical Juniper-mixed brush community.

per cent, a corresponding increase in the index for this flea began and continued until a maximum (34.4) was reached in December. In January the index decreased sharply to 6.4, and again increased to another peak (21.0) in April and May (see fig. 2).

The next most abundant species, *Hoplopsyllus anomalus* (Baker), was absent or negligible during most of the winter. The species index for *H. anomalus* increased gradually from April to a maximum (23.7) in August. The increment began when mean temperature increased above 64° F. and relative humidity decreased below 40 per cent. From July through September this species outnumbered *T. b. gladiolis*. Infestation by *H. anomalus* declined sharply when temperatures decreased below 64° F. and relative humidity increased to more than 40 per cent (see fig. 2).

A third species, *Rhadinopsylla heiseri* (McCoy), was recovered in moderate numbers during the winter months only. One specimen was collected in November and a total of 57 were recovered from six squirrels during December.

Other species collected are: One specimen each of *Pulex irritans* L., and *Foxella ignota utahensis* Wagner, and three specimens of *Orchopeas leucopus* (Baker). Because of their consistent recurrence on other hosts, these fleas are considered to be of accidental occurrence on antelope ground squirrels.

Figure 3 shows monthly averages of male and female *T. b. gladiolis* and *H. anomalus* per squirrel collected during this study. Average numbers of female *T. b. gladiolis* were higher than males throughout the year, except in September when the total number of this species was low. Female *H. anomalus* were more abundant than males in all months except April. In April the total number was just beginning a gradual increase following a four months' absence of this species from collections.

From a total of 107 female hosts examined during the investigation, the average infestation with fleas of all species was 15.7 per animal. Eighty-six (80%) of the females were infested with fleas. The average infestation of 109 males was 17.8 per animal. One hundred two (93%) of the males were infested. The ratio of infestation of the two sexes remained relatively constant seasonwise.

DISCUSSION.—These data indicate a seasonal correlation between abundance of *T. b. gladiolis* and *H. anomalus* which seems to be reversed in the two species, the former being more abundant during cool weather, the latter during warm weather. The data are inadequate for detailed specific conclusions since this would require a longer period of study, but from the data available it appears that the temperature of 64° F. and the relative atmospheric humidity of 40% represent approximate critical points at which changes in species indices begin to reverse for the two species considered.

Other environmental or hereditary factors may have been of significance in influencing the composition of the flea fauna, but no evidence is available to afford proper evaluations. Stewart and Evans (1941) reported that no correlation could be detected between California ground squirrel flea indices and either atmospheric or burrow temperatures, except that *Diamanus montanus* was predominant while mean temperature was below 75° F. and *H. anomalus* predominated while the mean temperature was above 75° F.

The occurrence of *T. b. gladiolis*, as indicated by seasonal variations in abundance on antelope ground squirrels, is similar to that of *Diamanus montanus* on California ground squirrels as reported by Holdenried *et al.* (1951), in which the flea populations from the ground squirrel, *C. beecheyi*, consisted mainly of the two species, *D. montanus*,

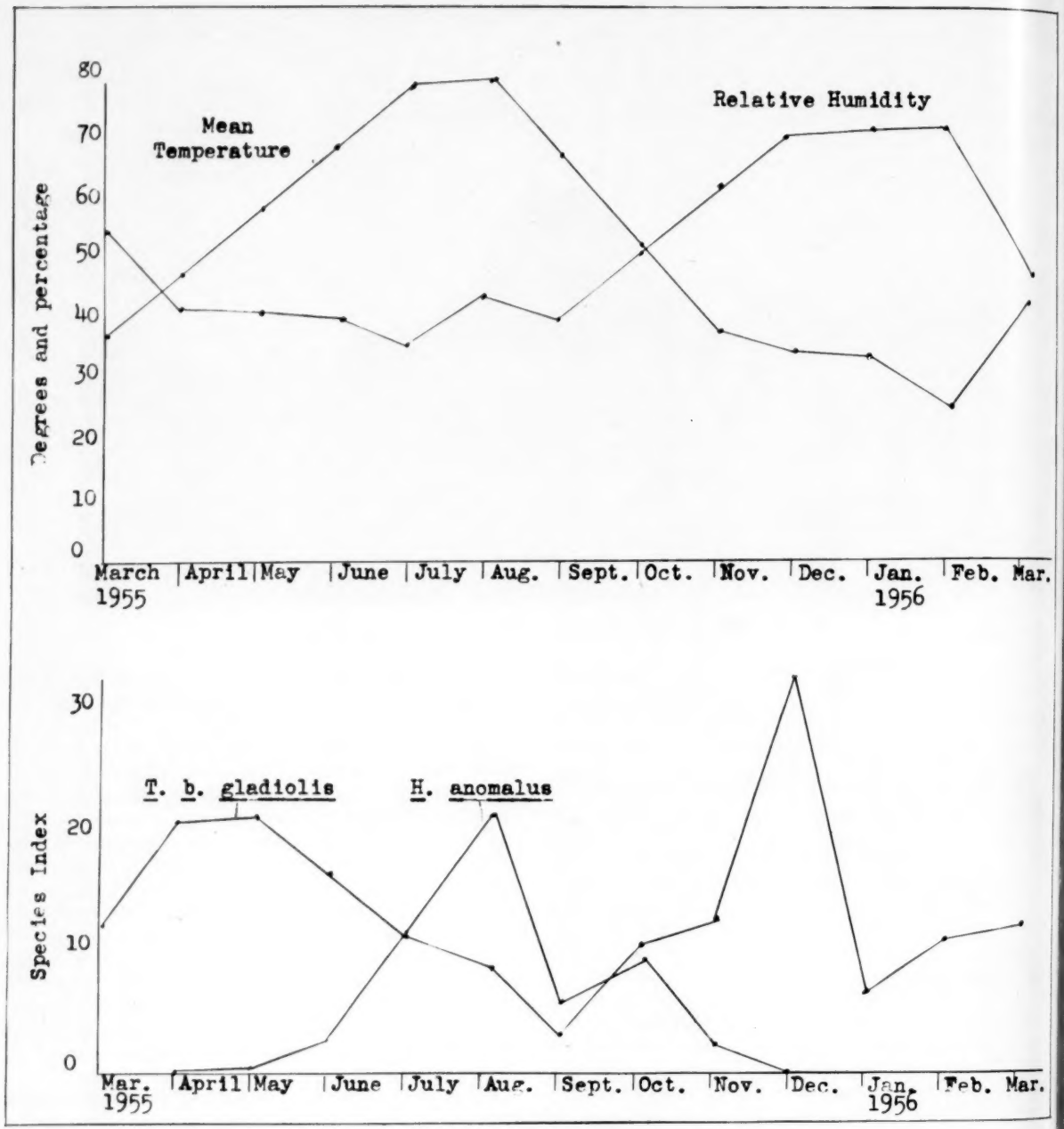


FIG. 2.—Seasonal occurrence of two species of fleas from antelope ground squirrels compared with temperature and relative humidity, March, 1955 to March, 1956.

and *H. anomalus*. The latter was most abundant during summer months, the former from October through March. Although the seasonal fluctuations in population dynamics of fleas in the two studies are similar, and both correspond to seasonal temperature changes in similar manner, the over-all seasonal index of fleas in this area remained consistently lower than in California. This possibly can be accounted for by climatic differences, size of host species, or by other ecological factors characterized by host environment.

The sudden decrease in the index of *T. b. gladiolis* during January cannot be correlated with either temperature or relative humidity conditions, since both remained relatively constant during that period. According to unpublished additional data accumulated over a four year period, 1951–1954, by members of Ecological Research, this species occurred on antelope ground squirrels more abundantly in January than in December. A decline in the index, as shown by the additional data, occurred in February. With the exception of the above mentioned differences, the seasonal indices for the two species were the same in both studies. It must be mentioned here that

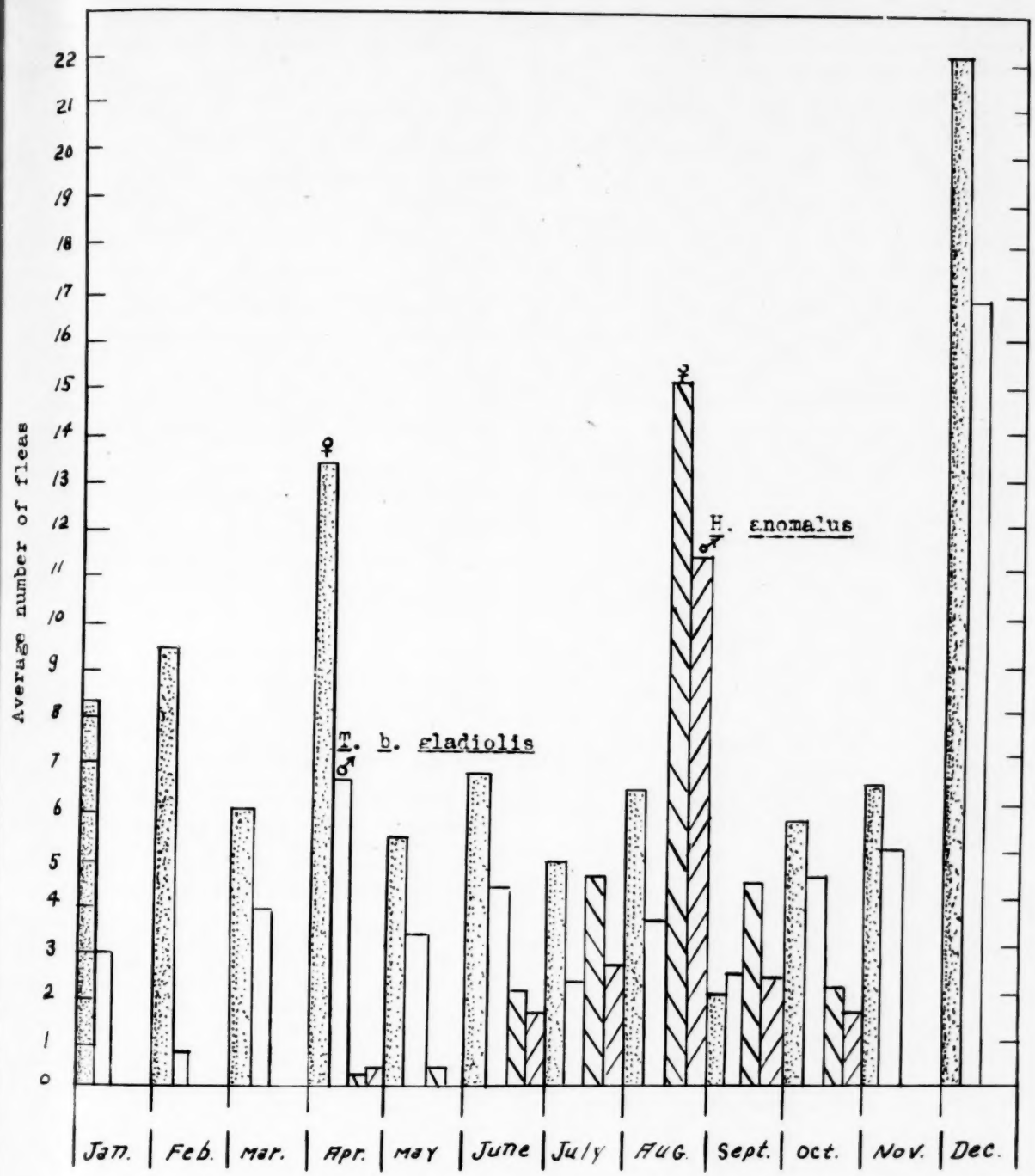


FIG. 3.—Seasonal variation in sex ratio of fleas from antelope ground squirrels.

the information gathered during 1951–54 was from hosts and fleas collected from various habitat types throughout a large area, and by many workers whose techniques for removing fleas from hosts varied. It is interesting to note that the seasonal fluctuations observed in the twelve month period have been substantiated by similar fluctuations occurring in other years. Similar findings were reported by Holdenried *et al.* (1951) with respect to their study of California ground squirrels.

The occurrence of *R. heiseri* on antelope ground squirrels in this area has previously been established from collections made by the Ecological Research staff, University of Utah. However, little evidence could be found from these collections to substantiate a correlative host preference for the host species. During that time eleven specimens had been collected from eight squirrels out of a total of 223 examined at various periods throughout each year. Two specimens were collected from the deer mouse,

Peromyscus maniculatus sonoriensis (LeConte), and one from the wood rat *Neotoma lepida lepida* Thomas. This flea had previously been recorded from *Citellus lecurus* in Inyo County, California, during November 1936 (Morlan & Prince, 1954). The possibility that *R. heiseri* is a nest inhabitor (spends most of the time in the nest) seems likely because of the comparatively small number taken from the hosts. However, none have been collected from seven squirrel burrows excavated in this area.

There was a high infestation rate of female fleas composing the flea fauna on antelope ground squirrels throughout the twelve-month investigation. A predominance of males of both *T. b. gladiolis* and *H. anomalus* occurred when the infestation rate of squirrels of each respective species was low. Males did not predominate in the flea population at any other time. In a seasonal study of mammal fleas from Santa Fe County, New Mexico, Morlan (1955) found that in several species the sex ratio showed a predominance of females, except during short periods of the year. In some instances the proportion of females decreased during the first or second month following the peak of seasonal abundance. In other species, males were predominant during certain months of one year, but not during the same month of a different year. Morlan suggested "observed changes in sex ratio may be primarily associated with seasonal changes in reproductive activity, which, in turn, are probably influenced by climatic conditions."

It was shown in this study that male squirrels were more heavily infested and a larger percentage of individuals were infested than females. The significance of these observations is not known, even though the findings corroborate results from investigations reported by Holdenried *et al.* (1951). However, the results of the studies jointly may reflect similarities in activity patterns of the two host species, and host-flea ecology within the two areas.

The wide distribution of the antelope ground squirrel offers excellent opportunities for a study of its ecological relationship with fleas under varied environmental conditions throughout its geographic range. Perhaps similar studies in other parts of the range will eventually indicate the extent to which ecological factors influence flea-host associations. Detailed studies concerning seasonal composition of ectoparasites from rodents which are known to be reservoirs or vectors of infectious diseases communicable to man or livestock, are of extreme importance. Information regarding these associations, when applied to hosts living in enzootic areas, potentially would shorten the time required for emergency control measures, or for survey programs to be effected. It is of utmost importance to know what time of year to survey or control seasonally influenced ectoparasites or other vectors.

SUMMARY.—Live trapping of antelope ground squirrels, *Citellus leucurus leucurus* (Merriam), during the period from March 1955 to March 1956 in the Great Salt Lake Desert showed that they were infested mainly with three species of fleas listed in order of abundance: (1) *Thrassis bacchi gladiolis* (Jordan), (2) *Hoplopsyllus anomalus* (Baker) and (3) *Radinopsylla heiseri* (McCoy).

T. b. gladiolis reached a maximum abundance on squirrels in December, and a minimum during the period June through September. Another peak in abundance of this flea occurred in April and May. *H. anomalus* was most abundant during the warm summer months, and was not collected during the period December through March. *R. heiseri* was collected mainly in December.

Initial changes in the indices of *T. b. gladiolis* and *H. anomalus* were correlated with mean monthly temperature readings of 64° F. and relative atmospheric humidity of 40 per cent; *T. b. gladiolis* predominated when average temperatures were below 64° F. and relative humidity was above 40 per cent. *H. anomalus* was more abundant when temperatures were above 64° F. and relative humidity was below 40 per cent.

A predominance of female fleas in both species was observed in all months, except when there was a low infestation rate for that species. Male antelope ground squirrels were more heavily infested than females.

REFERENCES CITED

Holdenried, R., F. C. Evans and D. S. Longanecker. 1951. Host-parasite-disease relationships in a mammalian community in the central coast range of California. Ecol. Monogr. **21**: 1–18.

Linsdale, J. M. 1946. The California ground squirrel. A record of observations made in the Hastings Natural History Reservation, Berkeley, xl+467 pp.

Miller, G. S., and R. Kellogg. 1955. List of North American recent mammals. U. S. Natl. Mus. Bull. **205**, 954 pp.

Morlan, Harvey B. 1955. Mammal fleas of Santa Fe County, New Mexico. Texas Repts. Biol. and Med. **13**(1) 93–125.

Morlan, Harvey B., and Frank M. Prince. 1954. Notes on the subfamily Rhadinopsyllinae Wagner 1930 (Siphonaptera, Histrichopsyllidae) and descriptions of a new species, (*Rhadinopsylla multidenticulatus*). Texas Rept. Biol. and Med. **12**(4): 1037–46.

Pollitzer, R. 1954. Plague. World Health Organization, 698 pp.

Stewart, M. A., and F. C. Evans, 1941. A comparative study of rodent and burrow flea populations. Proc. Soc. Exptl. Biol. and Med. 47: 140–2.

Woodbury, A. M., and D. D. Parker, 1954. Studies of tularemia, ***Pasteurella tularensis***, Ecol. Res., Univ. of Utah, Spec. Rept. No. **2**, 14 pp.

Laboratory Studies of the Life Cycle of the Carrot Weevil[1]

J. M. Wright[2] and G. C. Decker[3]

Abstract

In laboratory rearing studies on the carrot weevil, *Listronotus oregonensis* (LeC.), at Urbana, Illinois, during the summers of 1951 and 1952, the average duration of the life cycle from egg to adult in two generations each ranged from 33.5 to 41.1 days. With maximum longevity for adult females of 103 days for the first generation and 428 days for second generation extreme overlapping makes a clear demarcation of the two principal generations difficult.

When the carrot weevil, *Listronotus oregonensis* (LeC.), invaded the large northern Illinois canning carrot acreage in 1947, growers and researchers were confronted with a pest about which they knew relatively little. Although detailed life history studies of the weevil were reported in the literature, they appeared to be somewhat conflicting. Chandler (1926), from observations in the East St. Louis, Illinois, area, believed that the weevil was single brooded in Illinois. Harris (1926) studied what he called the carrot curculio, *Listronotus rudipennis* Blatchley, in the Burlington, Iowa truck gardening area and reported that there were probably three generations with continuous breeding throughout the summer. He found that the length of the life cycle from egg to adult averaged about 30.9 days. Boyce (1927) reared three generations on Long Island, with considerable overlapping of broods, and Pepper (1942) observed two full broods with a partial third brood in northern New Jersey and three full broods with a partial fourth brood in southern New Jersey. He reported such an overlapping of broods that all stages of the insect could be found in the field from late May until October.

Since detailed knowledge of the life cycle of a pest is fundamental in developing efficient and effective control measures, laboratory life history studies were conducted at Urbana, Illinois, during the summers of 1951 and 1952 in order to ascertain these details for the carrot weevil in northern Illinois.

Laboratory Methods.—On May 8, 1951, five male and two female weevils were collected and brought to the laboratory to begin these studies. Each female was confined with two males in a pint ice cream carton in which the cardboard disc in the top was replaced with wire screen. The weevils were fed pieces of fresh carrot which included approximately 1 inch of root and 1 inch of green stems. The female weevils readily oviposited in both stems and carrots. After oviposition began the food was changed daily and records kept of the number and locations of egg cavities and of the number of eggs in each cavity. The eggs were carefully dissected out of the carrots and stems under a microscope. As each egg was removed it was transferred with a moistened camel's-hair brush to a fresh carrot slice which had been prepared beforehand by punching six to ten small holes half way through the slice with a small stylus. These holes were placed in a circle near the outer edge of the slice. The eggs were placed one to a hole. This method simulated natural oviposition. As all the holes in a slice were filled it was placed in a 5-cm. petri dish on a moistened disc of filter paper. The eggs remained in place until hatching.

Upon hatching the young larvae were carefully dissected out of the carrot slice, placed on a fresh carrot slice and assigned a number. Each larva was removed from its food daily and given fresh food, note being made of the dates of molting in order to determine the number of larval instars and the duration of each. Mature larvae, when ready to pupate, left their food and even though fresh food was supplied, refused to feed further. This was considered the beginning of the prepupal stage. At this stage the larva was placed in a small glass crystallizing dish 5 cm. wide and 3.5 cm. deep, covered with soil moist enough to ball together when squeezed between the fingers, and allowed to pupate. Preliminary trials had shown that the pupa did not survive in very dry or very wet soil.

Records of the length of the prepupal stage were made by screening the soil in the pupating dishes to recover the pupal cell and carefully breaking away the end of the pupal chamber to observe the development of the individual within.

When the adult emerged it was immediately placed with another adult of the opposite sex, if available, and allowed to feed or oviposit as outlined above.

The technique in the 1952 studies was similar except that the adults were confined in 9.5-cm. petri dishes.

All rearing was at room temperatures. Adults kept over winter were placed individually in metal soil sample cans approximately the same size as the pupating dishes, covered with moist soil, and stored at 44 degrees F. in a refrigerator.

Results.—*Preoviposition period.*—There was considerable variation in the length of the preoviposition period of different weevils in these studies. The two overwintering females which were brought into the laboratory to begin the life cycle studies laid their first eggs 5 days after being placed with males. In all probability these females had already mated in the field and, although they had apparently laid no eggs, were partly through the preoviposition period. Two second generation females in 1952, numbers 43 and 100, underwent extended periods of 43 days before laying their first eggs. Quite consistent results were obtained with first generation adults in 1951 when preoviposition periods of five females ranged from 9 to 19 days, with an average of 13.2 days. Preoviposition periods of three first generation females in 1952 were 17, 19, and 34 days for a mean of 23.3 days. Pepper (1942) suggested that some females are fertilized before entering hibernation. This is indicated in these studies as female number 147, which had been placed with a male the previous fall, laid her first eggs only 2 days after being taken out of hibernation. (Table 1).

Oviposition.—The female deposits her eggs in small cavities which she chews in the stems, crown or exposed

[1] Data taken from thesis presented by the senior author to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the Doctor of Philosophy Degree.

[2] Formerly Associate Entomologist, Illinois Natural History Survey, now Extension Entomologist, University of Wisconsin.

[3] Entomologist and Head, Section of Economic Entomology, Illinois Natural History Survey and Illinois Agricultural Experiment Station, Urbana.

Table 1.—Preoviposition periods of some first and second generation carrot weevil females under laboratory conditions. Urbana, Illinois. 1951–52.

Female Number	Date of Mating	Date First Eggs Laid	Number of Days Mating to First Eggs
First generation			
20	June 21, 1951	July 9, 1951	19
45	July 8, 1951	July 20, 1951	12
81	July 28, 1951	Aug. 11, 1951	14
82	July 24, 1951	Aug. 2, 1951	9
102	July 30, 1951	Aug. 11, 1951	12
147	May 16, 1952	May 18, 1952	2[a]
8	July 11, 1952	July 30, 1952	19
16	July 7, 1952	Aug. 10, 1952	34
28	July 13, 1952	July 30, 1952	17
Second generation			
1	May 8, 1951	May 13, 1951	5[b]
2	May 8, 1951	May 13, 1951	5[b]
43	May 16, 1952	June 28, 1952	43
100	May 16, 1952	June 28, 1952	43

[a] Had been placed with male the previous fall but had laid no eggs.
[b] Brought in from field, apparently already mated.

root of the plant. In the present study it was observed that female weevils oviposited readily in either stems or roots but when both were available for oviposition the weevils showed a decided preference for ovipositing in the carrot itself. (Table 2).

In both years of the study approximately two-thirds of all eggs were laid in the carrots. (Table 2).

The proportions of cavities and eggs laid in stems and in carrots were remarkably similar in the 2 years. In 1951 all eggs were produced with both stems and carrots available. In 1952, however, less than half of the eggs were laid at times when both oviposition sites were available and a selection could be made. The number of eggs per cavity was slightly higher when cavities were made in the stems. This was true both years. The number of eggs per cavity ranged from 1 to 18.

The number of eggs per cavity also varied with individual females. Among the second generation females this average varied from 1.72 to 3.52 eggs per cavity. The range among first generation females was similar, being from 1.75 to 3.30 eggs per cavity. It also appeared that the number of eggs per cavity varied with the fecundity of the females. Among first generation females in 1951 there was direct correlation between total number of eggs laid and average eggs per cavity. (Table 3). Female number 81, which produced a total of 234 eggs, deposited an average of 2.52 eggs per cavity, while the most prolific female of this group, number 20, produced a total of 799 eggs with an average of 3.30 eggs per cavity. These relationships were not so evident in 1952, possibly due to the small numbers of eggs laid by most of the females.

Duration of stages.—These studies showed that the carrot weevil goes through five larval instars of approximately equal duration. (Tables 4 and 5.).

Table 2.—Number and proportion of cavities made and eggs deposited in carrots and stems by carrot weevil females under laboratory conditions. Urbana, Illinois. 1951–52.

Place of Oviposition	1951		1952[a]	
	Number of Eggs and Cavities	Per Cent of Total	Number of Eggs and Cavities	Per Cent of Total
Carrots				
Cavities	895	68.3	89	69.0
Eggs	2451	65.2	239	63.6
Eggs per cavity	2.74		2.69	
Stems				
Cavities	416	31.7	40	31.0
Eggs	1307	34.8	137	36.4
Eggs per cavity	3.14		3.43	
Total				
Cavities	1311		128	
Eggs	3758		376	
Eggs per cavity	2.87		2.91	

[a] Only those eggs laid when both root and stem were available for oviposition.

Table 3.—Total number of egg cavities made and eggs laid by first and second generation carrot weevil females under laboratory conditions. Urbana, Illinois. 1951–52.

1951				1952			
Female Number	Number of Cavities	Number of Eggs	Eggs per Cavity	Female Number	Number of Cavities	Number of Eggs	Eggs per Cavity
First generation							
20	242	799	3.3	8	4	7	1.8
45	159	519	3.3	16	3	8	2.7
81	93	234	2.5	28	7	15	2.1
82	154	424	2.8	147	166	585	3.5
102	163	535	3.3				
Second generation							
1	148	377	2.6	33	39	67	1.7
2	352	870	2.5	43	40	84	2.1
				100	11	22	2.0
Total	1311	3758	2.87		270	788	2.9

Table 4.—Duration of each stage of carrot weevil life cycle as determined from laboratory rearing. Urbana, Illinois. 1951.

Stage of Development	Duration of Stage in Days					
	First Generation			Second Generation		
	Min.	Max.	Mean	Min.	Max.	Mean
Egg	5	8	6.0	5	7	5.6
Larva						
First instar	2	5	2.6	2	5	2.6
Second instar	2	5	2.7	2	5	2.9
Third instar	2	6	2.8	2	7	3.2
Fourth instar	2	4	2.7	2	7	2.8
Fifth instar	1	8	3.4	1	7	3.3
Prepupa	3	6	4.8	3	6	4.1
Pupa	6	16	10.5	5	20	13.6
Egg to adult	30	49	36.2	30	53	41.1
Adult female	—	103	—		428	—

Table 5.—Duration of each immature stage of carrot weevil life cycle as determined from laboratory rearing. Urbana, Illinois. 1952.

Stage of Development	Duration of Stage in Days					
	First generation			Second generation		
	Min.	Max.	Mean	Min.	Max.	Mean
Egg	4	10	5.5	5	8	6.1
Larva						
First instar	2	6	2.7	2	3	2.2
Second instar	2	3	2.4	2	3	2.2
Third instar	2	4	2.7	2	5	3.2
Fourth instar	2	4	2.7	2	4	2.8
Fifth instar	1	10	2.6	2	5	2.7
Prepupa	2	6	3.1	2	6	3.0
Pupa	6	17	10.3	9	17	13.7
Egg to adult	27	44	33.6	30	43	35.6

Two generations of the weevil developed each year. In both years second generation forms required a longer period of time to complete their development than first generation forms. The differences were due chiefly to the second generation pupal period being approximately 3 days longer than the first generation pupal period. In 1952 the first generation prepupal period was nearly 2 days shorter and second generation prepupal period 1 day shorter than in 1951. Differences in duration of the other stages in the 2 years were only slight. Minimum, maximum, and mean duration of the various stages did not agree closely with those reported by Pepper (1942). However, the fact that the present rearing was done in the laboratory and in a different locale could account for these differences. There was no indication of a partial third brood in these studies as second generation females emerging from pupation as early as August 29, 1951 and September 14, 1952 laid no eggs.

In these studies some of the female weevils lived and laid eggs for long periods of time. (Table 4). It is thus easy to see why there is such overlapping of broods and why the life cycle is difficult to follow closely in the field. Maximum longevity of females was not determined in 1952 because the study was terminated at the end of that summer.

REFERENCES CITED

Boyce, A. M. 1927. A study of the biology of the parsley stalk weevil. Jour. Econ. Ent. 20(6): 814–21.

Chandler, S. C. 1926. The economic importance of the carrot weevil in Illinois. Jour. Econ. Ent. 19(3): 490–94.

Harris, H. M. 1926. A new carrot pest, with notes on its life history. Jour. Econ. Ent. 19(3): 494–6.

Pepper, B. B. 1942. The carrot weevil, *Listronotus latiusculus* (Bohe.), in New Jersey and its control. New Jersey Agric. Expt. Sta. Bull. 693. 20 pp.

A Study of Certain Acetals as Synergists for Pyrethrins or Allethrin in House Fly Sprays[1]

P. G. PIQUETT, B. H. ALEXANDER, and W. F. BARTHEL, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

ABSTRACT

Seventeen synthetic acetals were tested in space sprays by the Campbell turntable method against house flies, *Musca domestica* L., as candidate synergists with pyrethrins or allethrin. Nine compounds, all containing the 3,4-methylenedioxyphenyl group, were found effective at the concentrations used. Physical properties of all compounds are also given.

In a recent review Beroza & Barthel (1956) discussed the effect of the chemical structure of 3,4-methylenedioxyphenyl compounds on their synergistic activity with pyrethrins and allethrin against the house fly. They concluded that compounds containing ether, ester, acetal, sulfoxide, sulfone, and amide substituents are the most promising as synergists. One acetal derivative, prepared last year by Beroza (1956) and designated 2-(2-ethoxyethoxy)ethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde, was found to be so highly synergistic (Fales *et al.* 1956, 1957, Gersdorff *et al.* 1956, 1957) that it is now being produced commercially. Consequently, Barthel & Alexander (1957) prepared similar acetals with simpler side chains, and reported them to be of interest as synergists.

The present paper reports the physical properties of other members of this series and also of related acetals, together with the results of tests made with mixtures of them with pyrethrins or allethrin. The related acetals contain a 2,3- or 3,4-dimethoxyphenyl group instead of the 3,4-methylenedioxyphenyl group. Many workers have demonstrated the importance of the latter structure in forming compounds with synergistic activity, but further comparison with closely related alkoxy compounds seemed desirable. All compounds were prepared from the appropriate benzyl alcohols and vinyl ethers by the methods described by Barthel & Alexander (1957). The compounds and their properties are given in table 1.

Sprays of the adjuncts alone and in mixtures with pyrethrins or allethrin were prepared in 1:3 acetone-kerosene (Deobase). The mixtures contained 1 part of insecticide to 10 parts of adjunct. The concentration of the adjunct alone was 20 mg. per milliliter, of the pyrethrins mixtures 0.5 plus 5.0, and of the allethrin mixtures 0.25 plus 2.5. Similar mixtures were prepared with piperonyl butoxide as the adjunct.

The sprays were tested by the Campbell turntable method against the house fly (*Musca domestica* L.) in comparison with sprays containing the insecticide alone, 1.3 mg. per milliliter of acetone-kerosene for pyrethrins and 0.67 mg. for allethrin. Approximately 100 adult flies, $2\frac{1}{2}$ to $3\frac{1}{2}$ days in age, were used in each test.

The sprays containing the adjuncts alone caused little or no knockdown or mortality. Those containing the insecticides with or without the adjuncts caused complete knockdown; the mean mortality results obtained with them are given in table 2. The concentrations of the sprays containing the insecticides alone were such that for preliminary approximations an equivalent mortality would indicate for the mixture an increase in toxicity probably significant as judged from previous work by this method. Since the adjuncts were nontoxic, such an increase could be ascribed to synergism.

For the first nine compounds in the tables synergistic activity was indicated with each insecticide at these concentrations. Four of them—ENT 21576, 21612, 21618, and 21619—were sufficiently promising as compared with

[1] Accepted for publication July 22, 1957.

Table 1.—Chemical data on acetals, R′— —CH_2R

ENT No.	Empirical Formula	Name	R	R′ (CH_2R being 1-)	Yield (%)	Boiling Point °C.	Boiling Point mm. Hg	$n_D^{25°}$
21256	$C_{19}H_{32}O_4$	4-[1-(2-ethylhexyloxy)ethoxy]methyl-1,2-methylenedioxybenzene	$-OCH(CH_3)OCH_2CH(C_2H_5)C_4H_9$	3,4-CH_2O_2-	60	154	0.8	1.4891
21257	$C_{12}H_{14}O_4$	4-(1-ethoxyethoxy)methyl-1,2-methylenedioxybenzene	$-OCH(CH_3)OC_2H_5$	3,4-CH_2O_2-	79	112–116	1.1	1.5021
21274	$C_{12}H_{13}ClO_4$	4-[1-(2-chloroethoxy)ethoxy]methyl-1,2-methylenedioxybenzene	$-OCH(CH_3)OCH_2CH_2Cl$	3,4-CH_2O_2-	83	136–137	.7	1.5202
21275	$C_{14}H_{20}O_4$	4-(1-butoxyethoxy)methyl-1,2-methylenedioxybenzene	$-OCH(CH_3)OC_4H_9(n)$	3,4-CH_2O_2-	71	131	.6	1.4954
21276	$C_{14}H_{20}O_4$	4-(1-isobutyoxyethoxy)methyl-1,2-methylenedioxybenzene	$-OCH(CH_3)OCH_2CH(CH_3)_2$	3,4-CH_2O_2-	63	123	.8	1.4938
21576	$C_{16}H_{24}O_6$	4-{1-[2-(2-ethoxyethoxy)ethoxy]ethoxy}methyl-1,2-methylenedioxybenzene	$-OCH(CH_3)O(C_2H_4O)_2C_2H_5$	3,4-CH_2O_2-	60	172–180	.8	1.4941
21612	$C_{19}H_{30}O_6$	4-{[3-[1-(2-ethoxyethoxy)ethoxy]ethoxy}-2-methyl]propyl-1,2-methylenedioxybenzene	$-CH(CH_3)CH_2O-CH(CH_3)O(C_2H_4O)_2C_2H_5$	3,4-CH_2O_2-	70	[a]	.05	1.4862
21618	$C_{19}H_{28}O_6$	5-allyl-4-{1-[2-(2-ethoxyethoxy)ethoxy]ethoxy}methyl-1,2-methylenedioxybenzene	$-OCH(CH_3)O(C_2H_4O)_2C_2H_5$	{3,4-CH_2O_2- {5-CH_2=CH-CH_2-	70	174–210	.3	1.5022
21619	$C_{19}H_{28}O_6$	4-{1-[2-(2-ethoxyethoxy)ethoxy]ethoxy}methyl-1,2-methylenedioxy-5-propenylbenzene	$-OCH(CH_3)O(C_2H_4O)_2C_2H_5$	{3,4-CH_2O_2- {5-CH-CH=CH-	60	182–209	.3	1.5136
21289	$C_{20}H_{34}O_4$	3-(1-butoxyethoxy)methyl-1,2-dimethoxybenzene	$-OCH(CH_3)OC_4H_9$	2,3-$(CH_3O)_2$-	81	131–135	.6	1.4880
21290	$C_{19}H_{32}O_4$	3-(1-isobutylethoxy)methyl-1,2-dimethoxybenzene	$-OCH(CH_3)OCH_2CH(CH_3)_2$	2,3-$(CH_3O)_2$-	84	134–139	.7	1.4888
21291	$C_{13}H_{19}ClO_4$	3-[1-(2-chloroethoxy)ethoxy]methyl-1,2-dimethoxybenzene	$-OCH(CH_3)OC_2H_4Cl$	2,3-$(CH_3O)_2$-	55	140–145	.7	1.5126
21293	$C_{19}H_{32}O_4$	3-[1-(2-ethylhexyloxy)ethoxy]methyl-1,2-dimethoxybenzene	$-OCH(CH_3)OCH_2CH(C_4H_9)C_2H_5$	2,3-$(CH_3O)_2$-	85	161–163	.9	1.4821
21292	$C_{13}H_{22}O_4$	3-(1-ethoxyethoxy)methyl-1,2-dimethoxybenzene	$-OCH(CH_3)OC_2H_5$	2,3-$(CH_3O)_2$-	79	117	.8	1.4940
21501	$C_{19}H_{32}O_4$	4-[1-(2-ethylhexyloxy)ethoxy]methyl-1,2-dimethoxybenzene	$-OCH(CH_3)OCH_2CH(C_4H_9)C_2H_5$	3,4-$(CH_3O)_2$-	56	150–170	.7	1.4742
21502	$C_{13}H_{22}O_4$	4-(1-ethoxyethoxy)methyl-1,2-dimethoxybenzene	$-OCH(CH_3)OC_2H_5$	3,4-$(CH_3O)_2$-	46	128–134	.7	1.5041
21503	$C_{13}H_{19}ClO_4$	4-[1-(2-chloroethoxy)ethoxy]methyl-1,2-dimethoxybenzene	$-OCH(CH_3)OC_2H_4Cl$	3,4-$(CH_3O)_2$-	31	150–172	.7	1.5180

[a] Used a short path still; bath temperature 140°–150° C.

piperonyl butoxide to warrant further testing. These nine compounds were those containing the 3,4-methoxylenedioxyphenyl group (R′=3,4-CH_2O_2-). The other eight compounds, containing the dimethoxyphenyl group (R′=2,3- or 3,4-$(CH_3O)_2$-), were ineffective at these concentrations.

Table 2.—Per cent mortalities of house flies caused by sprays containing pyrethrins or allethrin with and without certain acetals.[a]

ENT No. of Acetal	Pyrethrins Plus Acetal (0.5+5.0 mg./ml.)	Pyrethrins Alone (1.3 mg./ml.)	Allethrin Plus Acetal (0.25+2.5 mg./ml.)	Allethrin Alone (0.67 mg./ml.)
21256	79[b]	63[b]	45	51
21257	56	65	60[b]	58[b]
21274[b]	74	63	71	58
21275[b]	85	63	66	58
21276[b]	69	63	61	58
21576	96	50	79	65
21612	88	37	93	55
21618	89	37	75	55
21619	87	37	89	55
21289	18	56	18	78
21290	16	56	13	78
21291	14	56	9	78
21292	15	56	14	78
21293	19	56	16	78
21501	6	34	2	30
21502	3	34	4	30
21503	1	34	3	30
Piperonyl butoxide	100[c]	58[c]	76[b]	62[b]

[a] Two replicates except as noted.
[b] Four replicates.
[c] Six replicates.

References Cited

Barthel, W. F., and B. H. Alexander. 1957. Screening tests of miscellaneous compounds containing the methylenedioxyphenyl structure. ARS—33–42.

Beroza, Morton. 1956. 3,4-Methylenedioxyphenoxy compounds as synergists for natural and synthetic pyrethrins. Jour. Agric. and Food Chem. **4**(1): 49–53.

Beroza, Morton, and W. F. Barthel. 1957. Chemical structure and activity of pyrethrin and allethrin synergists for control of the house fly. Jour. Agric. and Food Chem. **5**(11): 855–9.

Fales, J. H., O. F. Bodenstein, and Morton Beroza. 1956. Effectiveness against house flies of some 3,4-methylenedioxyphenoxy compounds as synergists for pyrethrins and allethrin. Jour. Econ. Ent. **49**(3): 419–20.

Fales, J. H., O. F. Bodenstein, and Morton Beroza. 1957. Evaluation of a 3,4-methylenedioxyphenyl acetal as synergist for pyrethrins and allethrin against house flies, mosquitoes, roaches, Japanese beetles. Soap & Chem. Spec. **33**(2): 79.

Gersdorff, W. A., P. G. Piquett, and Morton Beroza. 1956. Comparative synergistic effects of synthetic 3,4-methylenedioxyphenoxy compounds in pyrethrum and allethrin fly sprays. Jour. Agric. and Food Chem. **4**(10): 858–62.

Gersdorff, W. A., P. G. Piquett, and Morton Beroza. 1957. The intensity of synergism of some methylenedioxyphenyl acetals with pyrethrins or allethrin in house fly sprays. Jour. Econ. Ent. **50**(4): 406–9.

Insecticide Tests Against the Boll Weevil and the Bollworm at Tallulah, La., in 1956[1]

T. R. Pfrimmer, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*[2]

Abstract

In small-plot experiments with several insecticides applied as dusts and sprays for the control of the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)) conducted at Tallulah, Louisiana, during 1956, Guthion gave excellent boll weevil control in all tests at dosages from 0.25 to 0.6 pound per acre. Calcium arsenate dust, methyl parathion at 0.5 and 0.67 pound, EPN at 0.5 and 0.8 pound, and malathion at 2.05 and 3.81 pounds per acre were slightly less effective. Methyl parathion at 0.40 pound, Dipterex and Dow ET-15 at 0.5 pound, and calcium arsenate spray at 12.9 pounds per acre gave only fair control. DDT was included in most of these treatments. In one experiment endrin spray at 0.3 pound gave fair control, but endrin dust at 0.37 pound per acre was ineffective. In other experiments endrin, Thiodan, and heptachlor were ineffective at the dosages used.

Endrin and combination insecticides containing DDT gave good control of the bollworm. At the dosages used Thiodan and Guthion without DDT were comparable to calcium arsenate dust for bollworm control.

Four experiments were conducted at Tallulah, Louisiana, in 1956 to evaluate various insecticides for control of the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)). The cotton fleahopper (*Psallus seriatus* (Reut.)), the cotton aphid (*Aphis gossypii* Glov.), and spider mites (Tetranychidae) were not present in sufficient numbers to cause economic damage in any of the experiments.

All experiments were conducted in field plots arranged in randomized blocks, with four replications of each treatment. In experiments 1 and 2 each plot was 16 rows wide and of sufficient length to contain about 0.2 and 0.125 acre, respectively. In experiments 3 and 4 each plot was 12 rows wide and of sufficient length to contain about 0.1 acre.

All infestation records were made on the 10 middle rows in experiments 1 and 2, and on the eight middle rows in experiments 3 and 4. Yield records in experiment 1 were made on the 10 middle rows and in experiments 2, 3, and 4 on the eight middle rows. Boll weevil injury was determined by examining 100 squares and bollworm injury by examining 100 bolls in each plot. Each infestation record was made at five points in each plot, 20 squares or bolls being examined at a point. The points were chosen by the use of random numbers.

Most of the spray materials were emulsifiable concentrates, but the Dipterex was a 50% soluble powder, and the calcium arsenate was a high-suspension wettable powder. All applications were made with a high-clearance, self-propelled sprayer. For most of the insecticides three No. 2 cone-type nozzles per row were used to apply a total volume of 4 to 6 gallons per acre, but the calcium arsenate was applied with three No. 6 cone-type nozzles per row to give 10 to 12 gallons per acre.

Dusts were applied with single-nozzle rotary hand guns early in the morning or late in the evening when the air was calm. The heptachlor granules in experiment 2 were applied with a hand grass seeder. Applications of sprays and dusts were made at approximately 5-day intervals for all treatments except in experiment 4.

In experiments 2, 3, and 4 blanket spray applications were required at the end of the season to protect young bolls after the temporary seasonal employees returned to school. These applications were as follows:

Experiment	*Dates*	*Treatment (pounds per acre)*
2	September 29 and October 3	Methyl parathion 0.5+endrin 0.2
3	September 14	EPN 0.5+endrin 0.2
	September 29 and October 5	Methyl parathion 0.5+endrin 0.2
4	September 29 and October 5	Guthion 0.25+endrin 0.2

All records other than yield records were completed before the blanket applications were made.

The results of experiments 1, 2, and 3 are shown in table 1 and of experiment 4 in table 2. In the discussion of results given below all comparisons are in terms of significant differences.

In *experiment 1* Guthion-DDT gave fewer weevil-punctured squares than any of the other treatments except methyl parathion-DDT. Methyl parathion-DDT and EPN-DDT were better than Dipterex-DDT or endrin. Plots treated with Dipterex-DDT had a lower weevil infestation than the check or endrin-treated plots. The endrin treatment did not differ significantly from the check. All insecticide-treated plots had fewer bollworm-injured bolls than the check. All insecticides except Dipterex-DDT gave increases in yield of seed cotton over the check, with no differences between treatments except between EPN-DDT and Dipterex-DDT.

In *experiment 2* Guthion-DDT at 0.52 pound of each per acre gave better weevil control than malathion-DDT, Dow ET-15, Thiodan, endrin, or heptachlor. Guthion at 0.26 pound plus DDT and methyl parathion were better than Dow ET-15, Thiodan, endrin, or heptachlor. Calcium arsenate and malathion-DDT were better than Thiodan, endrin, or heptachlor. Dow ET-15 had a lower weevil infestation than heptachlor or the check. Thiodan, endrin, and heptachlor did not differ from the check. There were no differences between any of the insecticide treatments and the check in the percentage of bollworm-injured bolls.

Because of the greater variability of soil fertility and moisture within the experimental area, yield results did not mean so much in experiment 2. Although the increases due to the treatments were as great as 730 pounds of seed cotton per acre, none of the treatments differed significantly from any other.

[1] Presented at the meeting of the Cotton States Branch, Entomological Society of America, Birmingham, Ala., February 4–6, 1957. Accepted for publication July 22, 1957.

[2] The author was assisted in the field by G. L. Garrison, M. B. Bailey, Jr., E. H. Fisher, C. E. Forrest, Jr., M. A. McDonald, N. P. Mier, H. D. Morton, C. E. Powell, Jr., and J. V. Tarver.

Table 1.—Effectiveness of various insecticides against the boll weevil and bollworm in small-plot field experiments. Tallulah, La. 1956.

Insecticide (Pounds per Gallon in Spray Concentrates, Per Cent in Dusts)	Active Ingredient (Pounds per Acre)	Per Cent of—		Seed Cotton (Pounds per Acre)	
		Weevil-punctured Squares	Bollworm-Injured Bolls	Total	Gain Over Check
Experiment 1 (14 spray applications between July 30 and October 3)					
Untreated check	—	44	7.0	2018	—
Dipterex 50% S.P.+DDT 2[a]	2.00+0.50	34	2.6	2525	507
Guthion 2+DDT 2	0.24+ .48	16	3.4	2778	760
EPN 4+DDT 2	.48+ .48	25	2.3	3100	1082
Methyl parathion 4+DDT 2	.48+ .48	22	3.8	2715	697
Endrin 1.6	.29	47	3.3	2613	595
L.S.D. at 5% level	—	8	1.7	548	—
Experiment 2 (11 applications between July 31 and September 23)					
Untreated check	—	38	5.4	1325	—
Sprays:					
Malathion 5+DDT 2	2.05+0.52	20	1.8	2055	730
Guthion 2+DDT 2	0.26+ .52	15	2.4	1795	470
	.52+ .52	11	2.6	1665	340
Dow ET-15 2	.50	27	3.8	1685	360
Thiodan 2	.50	35	3.3	1430	105
Endrin 1.6	.30	35	2.8	1555	230
Dusts:					
Methyl parathion 5[b]	.67	17	1.8	1915	590
Calcium arsenate	13.84	19	4.6	1765	440
Heptachlor granules 2.5	0.51	37	3.3	1800	475
L.S.D. at 5% level	—	8.3	—	—	
Experiment 3 (13 dust applications between July 18 and September 10)					
Untreated check	—	60	24.9	2595	
Malathion 20+DDT 5	3.81+0.95	41	7.5	3310	715
Guthion 2.5	0.37	29	15.1	3110	515
5	.60	25	10.2	3210	615
EPN 5+DDT 5	.80+ .80	41	7.1	3285	690
Methyl parathion 2.5+DDT 5	.40+ .80	48	4.9	3155	560
Thiodan 3	.50	53	11.0	3125	530
Endrin 2+sulfur 40	.45+9.00	54	4.4	2875	280
Calcium arsenate	15.25	29	11.4	3470	875
L.S.D. at 5% level		9	6.0	345	

[a] 13 applications.
[b] 12 applications.

Table 2.—Effectiveness of Guthion against the boll weevil and bollworm at different intervals of application.[a] Tallulah, La. 1956.

Insecticide (Pounds per Gallon in Spray Concentrates, Per Cent in Dusts)	Active Ingredient (Pounds per Acre)	Applications		Per Cent of—		Seed Cotton (Pounds per Acre)	
		Interval (Days)	Number	Weevil-Punctured Squares	Bollworm-Injured Bolls	Total	Gain Over Check
Untreated check				53	24.0	2405	
Sprays:							
Guthion 2	0.53	5	13	12	10.0	3300	895
	.54	7	9	22	15.5	3120	715
	.53	10	7	28	11.5	3235	830
Endrin 1.6	.30	5	14	38	6.3	3080	675
Calcium arsenate WP[b]	12.9	5	13	38	17.0	3155	750
Dusts:							
Guthion 5	0.56	5	15	16	10.3	3210	805
	.62	7	10	35	9.5	3000	595
	.59	10	7	31	20.5	3040	635
Endrin 2+sulfur 40[c]	0.37+7.37	5	15	49	6.0	3240	835
Calcium arsenate	13.12	5	14	22	13.5	3335	930
L.S.D. at 5% level				8	7.4	336	

[a] Experiment 4 (Applications between July 18 and September 19).
[b] Dust in last four applications.
[c] Spray in last two applications.

In *experiment 3* Guthion and calcium arsenate gave better weevil control than any of the other materials, malathion-DDT and EPN-DDT gave better control than Thiodan or endrin-sulfur, plots treated with methyl parathion-DDT had a lower infestation than the check plots, and Thiodan and endrin-sulfur did not differ from the check. All the treated plots had less bollworm injury than the check. Endrin-sulfur and methyl parathion-DDT gave better bollworm control than Thiodan, calcium arsenate, or Guthion 2.5%. The EPN-DDT and malathion-DDT plots showed less bollworm injury than the one treated with Guthion 2.5%.

All the treatments except endrin-sulfur gave increases in yield of seed cotton over the check. The yield in the plots treated with calcium arsenate was greater than in those treated with Guthion 2.5% or Thiodan. The benefit gained from boll weevil control with the Guthion treatments was offset somewhat by the losses to bollworms, as reflected in the yields.

In *experiment 4* Guthion spray at 5-day intervals gave better boll weevil control than any other treatment except Guthion dust at 5-day intervals. Guthion dust gave better control at 5-day than at 7-day intervals, Guthion dust or spray at 10-day intervals, endrin-sulfur dust, endrin spray, or calcium arsenate spray. Plots treated with Guthion spray at 7-day intervals and calcium arsenate dust had fewer weevil-infested squares than those treated with Guthion dust at 7- and 10-day intervals, endrin-sulfur dust, endrin spray, or calcium arsenate spray. Guthion spray at 10-day intervals gave better control than endrin-sulfur dust, endrin spray or calcium arsenate spray. Guthion dust at 7- and 10-day intervals, endrin spray, and calcium arsenate spray were better than endrin-sulfur dust. Endrin-sulfur dust did not differ significantly from the check.

All insecticide treatments except calcium arsenate spray and Guthion dust at 10-day intervals had less bollworm injury than the check. Endrin-sulfur dust was better than calcium arsenste dust and spray, Guthion spray at 7-day intervals, or dust at 10-day intervals. Endrin spray was better than Guthion spray at 7-day intervals and dust at 10-day intervals, or calcium arsenate spray. Guthion dust at 7-day intervals had fewer bollworm-injured bolls than calcium arsenate spray or Guthion dust at 10-day intervals. Guthion spray at 5- and 10-day intervals and dust at 5-day intervals were better than Guthion dust at 10-day intervals.

All the treatments gave increases in yield of seed cotton over the check, but there were no significant differences between treatments. The Guthion sprays gave slightly better boll weevil control and slightly greater yields than the Guthion dusts at the same interval.

Reproductive Potential and Longevity of Two Relatively Isolated Field Populations of Insecticide-Susceptible House Flies[1,2]

FRED W. KNAPP and HERBERT KNUTSON,[3] *Kansas State College, Manhattan*

ABSTRACT

Flies collected on two relatively isolated ranches near Ellsworth and Wilmore, Kan., 110 miles apart had virtually no insecticide resistance. Field collections were brought to the laboratory and daily records maintained of eggs laid, longevity, eggs hatched, resulting pupation, and resulting emergence.

The Ellsworth flies, compared with the Wilmore flies, laid a substantially greater number of eggs earlier in life, except during the second and third days, principally because of (1) a greater number of eggs per female fly-day, but also because of (2) a greater survival of female parents earlier in life. But the Wilmore flies, eventually, almost caught up with the Ellsworth flies in egg production because of a substantially greater production of eggs per female fly-day later in life.

Hatchability was less in the Ellsworth population early in life primarily because of a high percentage of hatch of the Wilmore eggs during the second and third days. Hatchability was greater in the Ellsworth flies later in life. Totals for the 27 days of the parents' lives were not significantly different. Pupation of newly hatched larvae and emergence of pupae were lower in the Ellsworth population during the first two 9-day periods as well as for the entire 27-day period of the parents' lives.

The Ellsworth population produced 24.3 adult offspring per female parent as compared with 49.8 by the Wilmore population, primarily because of the relatively high adult survival, from egg to adult, of Wilmore eggs laid during the (1) second and third days and (2) during the 10- to 18-day period of the lives of the parents, which was when a substantial portion of the Wilmore eggs were produced.

Differences in the biology of various house fly populations with various histories of insecticidal treatments have been recorded on several occasions in the literature. Metcalf (1955) summarized results of studies by various workers with chlorinated insecticides involving different strains of house flies which had developed various degrees of resistance independently, and concluded that "there is little evidence of positive correlation between the factors responsible for biotic potential or vigor and specific insecticide resistance." Alterations in the biology of an insecticide-susceptible strain of house flies have been demonstrated after one treatment with dieldrin (Afifi & Knutson 1956). Ouye & Knutson (1957), using the same insecticide-susceptible strain, found reproductive potential and longevity to be altered following treatments

[1] *Musca domestica* L.

[2] Contribution No. 692, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan. Supported in part by the Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-560. Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the Master of Science Degree in Entomology, Kansas State College. Accepted for publication July 22, 1957.

[3] Graduate Research Assistant, and Professor of Entomology and Entomologist, respectively. Advice of C. C. Roan and laboratory assistance of Milton T. Ouye, Thomas M. Gray and Jerry Stegman are gratefully acknowledged.

Table 1.—Reproductive potential and longevity of Ellsworth (E) and Wilmore (W) populations of house flies.

Successive Periods (Days)		Egg Production per ♀	Egg Production Per Cent Total Eggs[c]	Longevity Per Cent Survival Actual over Theoretical, Cumulative ♀ Fly Days	Hatchability[a] Per Cent Eggs that Hatched	Pupation[a] Per Cent Hatched Larvae that Pupated	Adult Emergence[a] Per Cent: Pupa that Emerged	Adult Emergence[a] Per Cent: Survival from Eggs	Potential Adult[b] Progeny per ♀
1–9	E	135 *[d]	47	95.1 **	74 *	13 *	53 *	5.1 *	6.9 *
	W	85	30	90.8	82	30	70	17.2	14.6
10–18	E	140 *	48	88.2 **	80 *	19 *	72 *	10.9 *	15.3 *
	W	171	62	80.7	76	32	80	19.5	33.3
19–27	E	15	5	70.2 *[e]	60	28 *	82 *	13.8 *	2.1
	W	21	8	66.3	61	20	74	9.0	1.9
Total	E	290			71	20 *	73 *		24.3 *
	W	277			73	27	75		49.8

* Significant at 5% level.
** Significant at 1% level.
[a] Percentages based upon a constant number of eggs.
[b] Egg production × survival rate from eggs.
[c] Per cent total eggs produced each 9-day period.
[d] W > E through 3rd day.
[e] Approximately equal after 25th day.

of the larvae with malathion. Most of the strains used by various workers for this type of investigation have been laboratory strains, sometimes laboratory-selected, and nearly all had been removed from the field for many generations.

This study was initiated to determine how much biological variation may occur between two relatively in secticide-susceptible, isolated, field populations.

Materials and Methods.—One population was collected near Ellsworth, Kans., on a ranch at least 1½ miles from the nearest adjacent fly breeding area. The last possible contact with insecticides was in 1951, when DDT and chlordane were used on range cattle for biting flies but not within and around the barns where house flies would normally be exposed to a residual deposit. During the past 2 or 3 years the barn had been used almost exclusively to raise young calves. The flies collected were abnormally dark in color; determination of the species was confirmed by C. W. Sabrosky, who also noted the dark color.

The other population was collected near Wilmore, Kan., 110 miles from the Ellsworth collection site, by D. L. Matthew, Jr., on a cattle ranch at least 1 mile from the nearest adjacent breeding place. No insecticides had been used for at least 10 years.

The Ellsworth population had an LD_{50} of 3.7 ug. per fly and the Wilmore 2.7 ug. per fly, as determined by topical application with DDT. This indicated virtually no resistance in both populations as indicated by an LD_{50} of 3.0 in the KUN non-resistant strain maintained in our laboratory.

Collections consisting of several hundred flies, which constituted most of the adult flies available at that time, were made at each of the two locations and brought into the laboratory. Mass rearing was conducted to obtain 12 replications of 500 females and 500 males of each of the two populations. Techniques for counting eggs, measuring hatchability, pupal attainment, adult emergence, longevity, and statistical analysis of data were identical with that described by Ouye & Knutson (1957). Data were based on the first 27 days of the females' lives, since egg production was negligible thereafter. Daily records were maintained, but results were grouped into three successive 9-day periods, for convenience and because differences generally became apparent at about the 10th and 19th days.

Results and Discussion.—Results are presented in table 1. Overall egg production in the Ellsworth population was greater than that of the Wilmore population during the first 9-day period of the parents' lives (135 vs. 85 per ♀) although daily egg production was significantly less during the first 3 days. Egg production in the Ellsworth population was less during the second 9-day period as compared with the Wilmore population (140 vs. 171 per ♀). Total egg production was 290 per female compared with 277 per female, respectively, but this difference was not significant.

Longevity in table 1 is expressed in terms of per cent survival, based upon the actual number of female fly-days at the end of each successive 9-day period, as compared with the theoretical number, i.e., the number of female fly-days if all females had lived throughout each of the periods. Survival in the Ellsworth population was significantly greater at the end of the first and second 9-day periods (95.1 vs. 90.8 and 88.2 vs. 80.7, respectively), but after the 25th day populations were approximately equal.

In summary, the Ellworth population compared with

the Wilmore population laid a substantially greater number of its eggs earlier in life (47% vs. 30% during the first 9-day period), principally from the fourth through the ninth day. This was principally because of a greater number of eggs per female fly-day, but also because of a greater survival of these female parents during that period. But the Wilmore flies nearly caught up in egg production by the end of the second 9-day period (48 vs. 62%) because of a substantially greater production of eggs per female fly-day.

Because hatchability, pupation and adult emergence data in table 1 were based upon a constant number of eggs for both populations, they do not reflect the difference in egg production that occurred between the two populations but rather the relative survival between eggs and newly hatched larvae, between newly hatched larvae and pupae, between pupae and emerged adults, and between eggs and emerged adults.

Hatchability in the Ellsworth population was less during the first 9-day period (74 vs. 82%), primarily because of a hatch of Wilmore eggs of more than 90 % during the first 3 days (not shown in table 1) which was when a substantial portion of the Wilmore eggs during this period were laid. Hatchability was greater in the Ellsworth population during the second 9-day period (80 vs. 76%). Total hatchability was, eventually, approximately equal.

Survival between newly hatched larvae and pupae in the Ellsworth population was substantially less during the first two 9-day periods (13 vs. 30% and 19 vs. 32%, respectively), but greater during the third 9-day period (28 vs. 20%). The Ellsworth population had a lower total survival (20% vs. 27%).

Survival from pupa to adult in the Ellsworth population was less during the first two 9-day periods (53 vs. 70% and 72 vs. 80%, respectively) but greater during the third 9-day period (82 vs. 74%). The Ellsworth population had a lower total survival (73% vs. 75%).

Survival from egg to adult in the Ellsworth population was less during the first two 9-day periods (5.1 vs. 17.2% and 10.9 vs. 19.5%, respectively) but greater during the third 9-day period (13.8 vs. 9.0%).

The ultimate evaluation of differences in reproductive potential is given by considering not only the relative number of eggs produced, but the relative adult emergence from these eggs based upon the percentage of adults surviving from a given number of eggs. The production of potential adult progeny per adult female parent during the first 9-day period was only 6.9 in the Ellsworth population as compared with 14.6 in the Wilmore population. This occurred despite the greater production of eggs by the Ellsworth population (135 vs. 85 eggs per female) because of the relatively low survival from egg to adult (5.1 vs. 17.2%). This was especially true during the first 3 days of the parents' lives, when the Ellsworth population was greatly exceeded by the Wilmore population in egg production at a time when the survival from egg to adult was a high 29.7% (not shown in table 1). During the second 9-day period, the Ellsworth population again produced considerably less potential adult progeny (15.3 vs. 33.3), because of a lower egg production (140 vs. 171 per female) and survival from egg to adult (10.9 vs. 19.5). During the third 9-day period, the potential adult progeny (2.1 vs. 1.9) was not significantly different. The total potential adult progeny of the Ellsworth population was less than half that of the Wilmore population (24.3 vs. 49.8 adult progeny per female parent).

Evidence of further differences between populations is shown when comparing the data obtained in this study with those of Ouye & Knutson (1957) in which they used an insecticide-susceptible but laboratory-reared strain. Using identical techniques, they found the potential adult progeny per female parent was 205.

The work reported herein may explain some of the variations in results of control measures, when presumably the same control techniques were used. It also emphasizes the innate differences between field populations and between strains, so that correlation between biological variations and such characteristics as resistance need not necessarily be expected.

REFERENCES CITED

Afifi, Saad E. D., and Herbert Knutson. 1956. Reproductive potential, longevity and weight of house flies which survived one insecticide treatment. Jour. Econ. Ent. 49(3): 310–3.

Metcalf, R. L. 1955. Physiological basis for insect resistance in insecticides. Physiol. Revs. 35(1): 197–232.

Ouye, Milton T., and Herbert Knutson, 1957. Reproductive potential, longevity, and weight of house flies following treatment of larvae with malathion. Jour. Econ. Ent. 50(4): 490–3.

Severe T_3 Strain of Tristeza Virus Transmitted by *Aphis gossypii* Glover[1]

PAUL A. NORMAN, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

In Florida *Aphis gossypii* Glover, *A. spiraecola* Patch, and *Toxoptera aurantii* (Fonsc.) have been found capable of transmitting the mild T_1 and T_2 strains of tristeza virus to citrus (Norman & Grant 1956). Recent tests show that *A. gossypii* is also capable of transmitting the severe T_3 strain of this virus (Grant & Higgins 1957). The sources of inoculum used were potted seedlings of pineapple sweet orange, Cleopatra mandarin, rough lemon, and *Tangarina cravo* that had been tested for presence of this strain following their inoculation by means of tissue grafts. Large colonies of these aphids were fed on the source plant for 24 hours, and then 300 aphids were transferred to Key lime indicator plants for a minimum of 6 hours for transmission feeding. Symptoms characteristic of the T_3 strain of tristeza appeared on 11 of 16 indicator plants in 94 days.

REFERENCES CITED

Norman, Paul A., and Theodore J. Grant. 1956. Transmission of tristeza virus by aphids in Florida. Florida State Hort. Soc. Proc. 69: 38–42.

Grant, Theodore J., and Richard P. Higgins. 1957. Occurrence of mixtures of tristeza virus strains in citrus. Phytopath. 47(4): 272–6.

[1] Accepted for publication November 7, 1957.

Drone Brood in Honey Bee Colonies[1]

M. Delia Allen, *Bee Research Department, Marischal College, Aberdeen, Scotland*

Abstract

The numbers of sealed drone cells produced in seven honey bee colonies were counted at intervals throughout the period of their production. On the same days measurements were made of the amounts of total brood in these colonies, and the spread of the two sets of curves was compared. It is shown that the period when there was considerable drone brood present had only about half the duration of the corresponding period for total brood. The number of sealed drone cells in individual colonies differed considerably and was apparently not related to the formation of queen cells in preparation for swarming. In the five colonies which made swarm preparations the interval of time between the start of the large scale production of drone cells and the laying of the first eggs in queen cells was subject to rather wide variation, but had a mean duration of about 3 weeks.

The annual cycles of brood rearing (Nolan 1925, Allen & Jeffree 1956), and numbers of bees (Farrar 1931, Jeffree 1955) in honey bee colonies are now fairly well established, but the same is not true of the production of drone brood. Huber (1792) suggested that the major period of drone egg laying commenced about 3 weeks before queen cells were formed, but few actual data have been presented.

In order to fill this gap the observations described in this paper were carried out.

Methods.—Preliminary estimations of the amount of sealed drone brood and of total brood were made on two colonies in 1952 by L. Murray, and in 1956 the author made more comprehensive measurements on five colonies in the departmental apiary. Sealed drone cells were counted individually and the amount of total brood was estimated by means of a specially constructed grid calibrated in sq. ins. (Jeffree 1955) which was placed over the comb. The term "total brood" here includes all worker and drone unsealed and sealed brood (including eggs).

The number of bees in each colony was determined at the time of maximum brood rearing (1) in 1952 by a weighing technique (Jeffree 1955), and (2) in 1956 by comparison with calibrated photographs (Jeffree 1951). The results are given in table 1A. Colony S.20 had a slight infection of Nosema disease in April, 1952, but other microscopical examinations (approximately 20 bees per sample) of this colony and of S.17 revealed no *Nosema* spores. All five colonies studied in 1956 were free from the disease when brood measurements were begun but some spores were later found in three of these colonies, though not in large numbers (table 1B).

When swarm preparations started (i. e., eggs present in queen cells) some manipulations were necessary as countermeasures, and details of these are given in table 2. In cases where the colony was sub-divided the quantity of brood in each part was measured, giving a total for the whole colony.

Results.—Figure 1 shows the mean number of sealed drone cells and the mean quantity of total brood present in the seven colonies studied in 1952 and 1956. For comparison, the relevant part of a curve of mean total brood of a large number of colonies in the departmental apiary during the years 1945–53 (Allen & Jeffree 1956) is also given. It illustrates that the mean total brood curve for the seven colonies here studied follows essentially the same course as that given by the earlier more extensive figures.

It is fairly evident from an inspection of figure 1 that the period during which there is considerable drone brood in the colonies is shorter than the corresponding period for total brood. The spread of a normal curve is usually measured at its points of inflection but since this convention cannot very well be applied in the present case the greater spread of the curve of total brood has been illustrated by drawing ordinates to intersect each curve at points equal to one-third of the respective maxima. The period of maximum production of total brood defined in this way extended over 108 days (7th May - 23rd August), while the corresponding period for the sealed drone brood had only about half this duration, being 50 days (11 th June - 31st July).

At the peak of the sealed drone brood curve the number of cells was 1,590, and it may be calculated that the maximum amount of total brood was 31,720 cells, using the figure of 26 cells per square inch given by Nolan (1925).

Figure 2 shows the values obtained for the individual colonies. It can be seen that there is relatively little varia-

[1] Accepted for publication July 25, 1957.

Table 1.—Colony sizes and Nosema disease.

Date	Colony No. S.17	S.20	5	6	17	34	39	Mean
			A. Number of bees in colony					
June 28	26,760	29,600	23,580	31,920	21,720	33,720	13,890	25,820
			B. Nosema					
April 18	−	+	−	−	−	−	−	
June 8	−	−	+	−	−	+	−	
July 8	−	−	−	−	+	−	−	

− *Nosema* spores absent.
+ *Nosema* spores present.

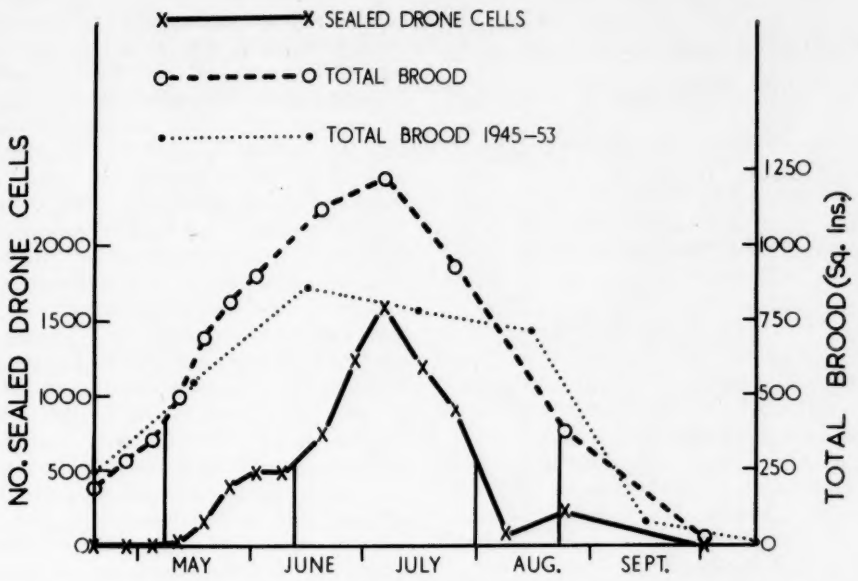


FIG. 1.—Mean quantity of drone brood in relation to total brood in seven colonies.

bility between the courses of the total brood curves, whereas the drone brood curves exhibit several minor fluctuations and in some cases noticeably differ one from another.

It has been suggested that there is a relationship between the date when the main production of drone brood begins and the date of formation of queen cells (Huber 1792), and this point was also examined. Taking the presence of a somewhat arbitrary figure of 50 sealed drone cells as a sign that large scale drone production had commenced it was possible to ascertain the lapse of time between then and the laying of the first eggs in queen cells (table 3).

It appears from these results that Huber's suggestion of 3 weeks as the length of this period has some foundation, although there is considerable variation in the interval before the queen lays in queen cells. However, it must be stressed that drone brood production is a virtually universal event in honey bee colonies, whereas the formation of queen cells is not, and therefore the presence of drone brood cannot be taken as a definite indication that queen cells will be formed later.

DISCUSSION.—Nolan's (1925) results give some indication that the start of brood rearing may be delayed by a cold spring, and Jeffree (unpublished) found a considerable displacement of the brood curve in the exceptionally late year 1947 at Aberdeen. However, in the present instance it has already been suggested that the available evidence indicates that brood rearing was not to any major extent either early or late in 1952 and 1956 and it would seem likely that the drone curve, which might be similarly influenced by very early or late seasons, was also in approximately the normal position.

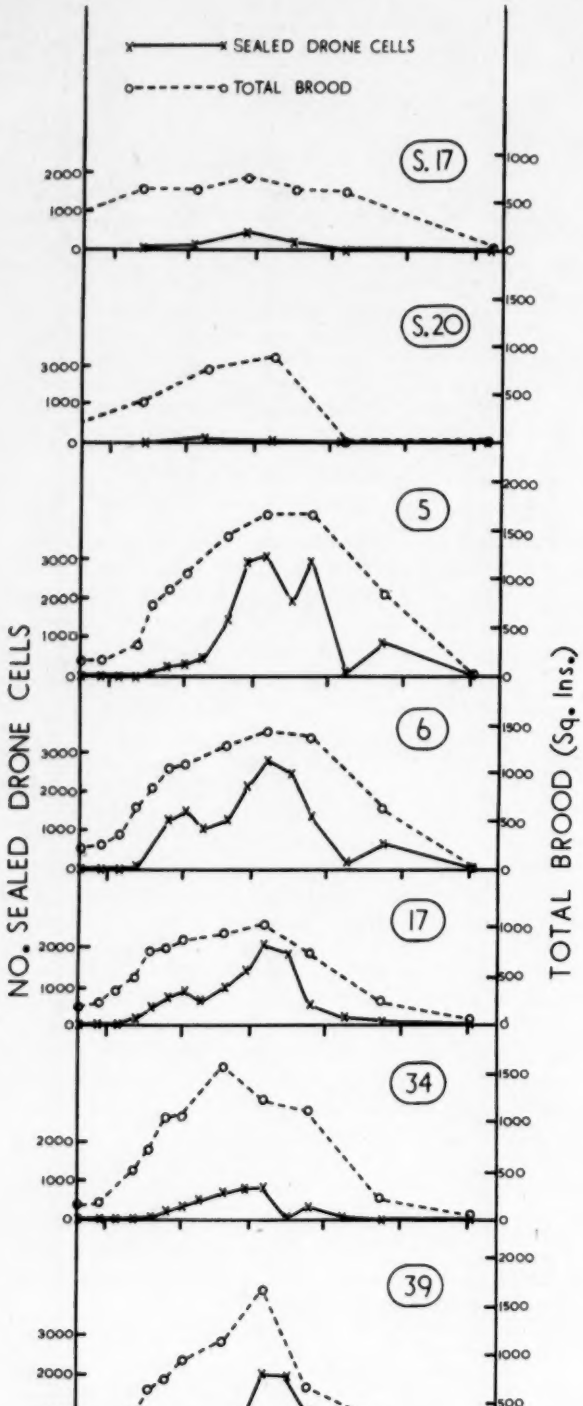
The timing of the rearing of drone brood in relation to swarming is of interest. The five colonies which made swarm preparations commenced them during the first 3 weeks of June (table 3), by which time a substantial

Table 2.—Details of swarm-prevention measures

No. of Colony	Manipulations
S.17	1) Empty combs inserted between brood combs (June 16, 1952)
	2) Divided into two sections by Snelgrove board: bottom part with old queen, top part allowed to rear new queen (June 26, 1952)
S.20	Divided into two sections by Snelgrove board: bottom part with old queen, top part allowed to rear new queen (June 20, 1952)
5	None
6	Empty combs inserted between brood combs (June 19 and 28, 1956)
17	1) Empty combs inserted between brood combs (June 1 and 19, 1956)
	2) Divided into two parts: five-frame nucleus allowed to rear new queen, and main stock with old queen (July 25, 1956)
34	1) Empty combs inserted between brood combs (June 19, 1956)
	2) Divided into two sections by Snelgrove board: bottom part allowed to rear new queen, top part with old queen (June 28, 1956)
39	Divided into three parts and each allowed to rear new queen (July 25, 1956)

Table 3.—Relationship between first large scale production of drone brood and queen cell formation.

No. of Colony	Earliest Date When 50 Sealed Drone Cells Were Present	Date of First Eggs in Queen Cells	Time Interval (days)
S.17	May 16, 1952	June 11, 1952	26
S.20	May 31, 1952	June 16, 1952	16
5	May 14, 1956	No queen cells	—
6	May 9, 1956	June 19, 1956	41
17	May 7, 1956	June 1, 1956	25
34	May 16, 1956	June 5, 1956	20
39	May 18, 1956	No queen cells until queenless (on July 18, 1956)	—



number of sealed drone cells was present. The amount of drone brood per colony does not, however, appear to be related to swarm preparations in the colony since colony No. 5, which had no eggs or larvae in queen cells at any stage, produced a large number of sealed drone cells. Similarly, colony No. 39 had very appreciable amounts of sealed drone brood in June and early July but no fertile queen cells until 18th July, when it became queenless.

Rosser (1934) found that colonies may vary greatly in their production of drone brood, and the differences in total amounts found in the colonies studied here are in agreement with this. Nolan (1925), in his presentation of curves of total brood, reported considerable irregularities which he attributed chiefly to the influence of weather factors and the variable intakes of pollen and nectar. In the present instance, curves for sealed drone cells in individual colonies showed fluctuations of similar extent, and it is probable that the same factors may have been responsible. In fact, it is well known that the workers will remove drone brood at times of food shortage and in bad weather (Langstroth 1890). A further factor almost certainly affecting the amount of drone brood produced is the total area of drone comb available for laying, and it has long been recommended in beekeeping practice that the proportion of drone comb should be kept to a minimum in order to reduce the amount of drone brood reared (Langstroth 1890).

ACKNOWLEDGMENT.—The author wishes to thank Mr. E. P. Jeffree for his advice and criticism during the course of this work.

REFERENCES CITED

Allen, M. Delia, and Jeffree, E. P., 1956. The influence of stored pollen and of colony size on the brood rearing of honeybees. Ann. Appl. Biol. **44:** 649–56.

Farrar, C. L. 1931. A measure of some factors affecting the development of the honey bee colony. Ph.D. Thesis at the Massachusetts State College.

Huber, F. 1792. Nouvelles observations sur les abeilles, I. Transl. (1926) Hamilton, Ill.: Dadant.

Jeffree, E. P. 1951. A photographic presentation of estimated numbers of honeybees (*Apis mellifera*, L.) on combs in 14×8½ inch frames. Bee World, **32**(12): 89–91.

Jeffree, E. P. 1955. Observations on the decline and growth of honey bee colonies. Jour. Econ. Ent. **48:** 723–6.

Langstroth, L. L. 1890. The hive and the honeybee. Rev. edn. Hamilton, Ill.: Dadant.

Nolan, W. J. 1925. The brood-rearing cycle of the honey bee. U. S. Dept. Agric. Bull. **1349:** 1–56.

Rosser, J. H. 1934. On drone rearing. Bee World **15:** 58.

FIG. 2.—Drone brood and total brood in seven colonies.

Biological and Chemical Oxidation of Tetramethyl Phosphorodiamidic Fluoride (Dimefox)[1]

B. W. Arthur and J. E. Casida, *Department of Entomology, University of Wisconsin, Madison*

Phosphoramides showed early promise as systemic insecticides for plants (Schrader 1947). Although other types of phosphorus compounds have largely replaced phosphoramides as systemic insecticides, schradan and dimefox are still of interest because of their selective toxicity (O'Brien & Spencer 1953, Krueger & Casida 1957), chemical and biological oxidative mechanisms (Heath *et al.* 1955, Tsuyuki *et al.* 1955, Spencer & O'Brien 1956) and high efficiency for certain practical insect control problems (Ripper *et al.* 1950, Michelbacher & Oatman 1955, Hanna *et al.* 1956). Comparative studies on schradan and dimefox have been reported concerning toxicity and pharmacological action in mammals (Okinaka *et al.* 1954), systemic activity in plants (David 1952) and insecticidal properties (David & Gardiner 1951, David 1952). Metabolic activation in mammals occurs with both dimefox and schradan to form more potent anticholinesterase agents (Aldridge & Barnes 1952). The activation (Fenwick *et al.* 1957) and detoxification (Okinaka *et al.* 1954) of dimefox by rat liver slices have been studied. Plant and insect metabolism of schradan has been frequently investigated. The biological and chemical properties of hexamethylphosphoramide, a related compound lacking the acid anhydride bond, have also been compared with schradan (Heath *et al.* 1952, 1955, Adkins *et al.* 1955).

This study concerns radiotracer experiments on the biological distribution and metabolism of dimefox compared with schradan and hexamethylphosphoramide.

Materials and Methods.— *Synthesis and Characterization of Radioactive Materials*—Red phosphorus-32 (4 weeks A.E.C. service irradiation at 7×10^{11} n/cm^2/sec flux) was chlorinated to form phosphorus trichloride (Forbes *et al.* 1946) and equimolar water was added in the presence of chlorine to form phosphorus oxychloride (Vanscheidt & Tolstopystov 1920). The phosphorus oxychloride was converted to tetramethylphosphorodiamidic chloride by reaction with four molar equivalents of anhydrous dimethylamine in anhydrous ether (Cook *et al.* 1949). This phosphorodiamidic chloride was then reacted with 3.6 molar equivalents of potassium fluoride in aqueous solution to form the tetramethyl phosphorodiamidic fluoride (Pound 1953), or with two molar equivalents of anhydrous dimethylamine to yield hexamethylphosphoramide (Heath & Casapieri 1951). Chromatography of the phosphorodiamidic fluoride reaction mixture on silica gel with an 80% chloroform and 20% *n*-hexane elutrient yielded about 3% schradan and 97% dimefox in two distinct peaks. Rechromatography of these materials yielded fractions which were identical to known compounds based on chromatography, partition distribution, and infrared spectrograms. Both schradan and dimefox were over 99% chemically and radiochemically pure. Hexamethyl-phosphoramide was chromatogramed using a chloroform elutrient and silica gel and was over 97% pure. Specific activity for the three materials was about 300 cpm/γ.

In vivo Distribution and Metabolism Studies—Insects.—The distribution of radioactive dimefox and schradan among the organ systems of adult female American cockroaches, *Periplaneta americana* (L.), was studied following topical application to the pronotum of 50γ insecticide in 20 μl. acetone per gm. of insect. Two replicates of eight cockroaches each were treated with either radiolabeled material and dissections made after holding the treated cockroaches 1 hour at 28° C. No symptoms of poisoning were apparent at the time of dissections. Techniques for dissection of cockroaches, weighing of tissues, determining the p.p.m. of total insecticide and metabolites and the percentage hydrolysis have been described (Arthur & Casida 1957) (Table 1).

For cockroach metabolism experiments, 50 to 100 adult females were injected in the hind femora with 1000 γ of radioactive dimefox or schradan per gm. of insect and held at 28°C. One hour after treatment when poisoning symptoms had just become evident, the insects were macerated in 100 ml. cold water with a Waring blendor and the resulting brei filtered through cheesecloth. This filtrate was extracted three times with equal volumes of chloroform which were combined, dried with anhydrous sodium sulfate and concentrated under reduced pressure. The cockroach residue after extraction contained 30% of the total radioactivity with dimefox and 9% with schradan, while the aqueous layer contained 24% and 17%, and the chloroform extracts 46% and 74% with dimefox and schradan, respectively. The chloroform extractables were then chromatographed and the fractions analyzed for total radioactivity, partition distribution between chloroform and water, anticholinesterase activity using whole human blood as the source of enzyme (Casida *et al.* 1956), and total formaldehyde formed on treatment with acid (Krueger 1949, Casida *et al.* 1954). In some instances, the organophosphates eluted from the column were also subjected to infrared analysis. Because of the possible instability of certain oxidized metabolites, the entire experiment from treating the cockroaches to completion of the chromatography and analysis was accomplished within a 24-hour period.

Mammals.—Tissue distribution studies were made on 200 gm. male white rats treated subcutaneously with 10 γ of radioactive dimefox per gm. of rat (about twice the LD_{50} value by this route of administration). At the time of death, (5 to 6 minutes after treatment), various tissues were removed, macerated in a minimum of cold water with a Waring blendor or mortar and pestle and the brei filtered through cheesecloth. The total p.p.m. of dimefox plus phosphorus-containing metabolites was calculated from the radioactivity in the aqueous filtrate of the tissue homogenates plus the non-water soluble radio-

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation and by the Division of Biology and Medicine of the U. S. Atomic Energy Commission. Accepted for publication July 26, 1957.

Table 1.—Distribution and stability of the absorbed radioactive dimefox and schradan in adult female American cockroaches 1 hour after topical application of 50γ/gm. to the pronotum.

Tissue	P.p.m. Based on Total Radioactivity[a]				Per Cent Hydrolysis	
	Dimefox		Schradan		Dimefox	Schradan
Salivary glands	26	18	39	35	60	14
Foregut	12	9	22	20	57	10
Crop	6	5	40	47	56	12
Gastric caeca	43	44	25	16	86	15
Midgut	12	12	31	37	89	17
Malpighian tubules	66	57	33	48	97	3
Hindgut	29	53	81	88	63	14
Nerve cord	31	30	25	42	61	2
Muscle	7	8	22	25	23	10
Fat body	45	42	16	13	82	17
Reproductive organs	14	12	15	28	59	5
Tracheae	8	8	9	13	27	0
Head	10	12	27	27	75	22
Pronotum	2	3	16	15	65	17
Cuticle	36	41	7	7	65	2
Residue	26	27	80	52	—	28

[a] Values are shown separately for the two replicates of eight cockroaches each.

activity remaining in the tissue residues (table 2). Percentage hydrolysis of the radiolabeled material was calculated from the partition coefficient, chloroform/water.

Mammalian excretion studies were made on 200 gm. male rats (3 replicates of 2 rats each) by treating orally with 2.5 γ of schradan or dimefox per gm. of rat. These oral dosages of schradan and dimefox produced no poisoning symptoms. Rats were held in metabolism cages which allowed for the separation of feces and urine (Comar 1955). Urine and feces were collected at periodic intervals and the total radioactivity and percentage insecticide hydrolysis determined (table 2). The rats were sacrificed 5 days after treatment and the total radioactivity remaining in several tissues was determined.

For studying the *in vivo* activation of dimefox, 17 male rats were treated subcutaneously with 10 γ of radioactive dimefox per gm. of rat. At the time of death the livers were removed and extracted. Chloroform-soluble materials from the livers were fractionated on silica gel and the anticholinesterase activity and partitioning properties of the eluate fractions compared with those of unmetabolized dimefox (table 5).

Plants.—Seedling Perfection pea plants and Fordhook bean plants were utilized in distribution and metabolic studies of radioactive dimefox and hexamethylphosphoramide, respectively. Plants were placed in an aqueous solution containing 2000 p.p.m. of either radiolabeled material for 24 hours at 28° C. after which the plants were transferred to a complete nutrient solution containing no insecticide. At various intervals after removing the insecticide source, samples of 12 plants each were divided into roots, stems and leaves and the total radioactivity in the parts determined. Plant parts were homogenized in water and a portion of the aqueous layer partitioned with an equal volume of chloroform and the percentage hydrolysis calculated. Exhaustive partitioning (Heath *et al.* 1952) between chloroform and water was then utilized to indicate the presence of non-hydrolyzed metabolites in the plant extracts. The chloroform-soluble radiation recovered by three extractions of the plant brei with equal volumes of chloroform was chromatographed on silica gel and total formaldehyde content determined for the fractions and infrared spectrograms obtained for the metabolites.

In vitro Metabolism Studies.—*In vitro* metabolism of radioactive dimefox or hexamethylphosphoramide by rat liver slices was studied using a method described by DuBois *et al.* (1950) for producing the schradan metabolite. After incubation of the amides with rat liver slices in an oxygen atmosphere for 4 hours, the buffered aqueous medium (pH 7.2) was extracted five times with equal volumes of chloroform, the combined chloroform extracts concentrated and chromatographed on silica gel. The chromatographic fractions were analyzed for phosphorus colorimetrically (Allen 1940) or radiometrically, change in total phosphorus partitioning properties, anticholinesterase activity, and content of formaldehyde. Infrared spectrograms were obtained on combined column fractions.

The ability of human serum esterases to hydrolyze the phosphoramides was also investigated (Method of Arthur & Casida 1957).

Chemical Oxidation of Dimefox.—The products from oxidation of dimefox with peracetic acid, potassium permanganate, and hydrogen peroxide were studied using the procedures of Tsuyuki *et al.* (1955). Dimefox was reacted for 3 hours at 28° C. with equimolar peracetic acid in chloroform or with equimolar permanganate in water. Superoxol (30% H_2O_2) oxidation of dimefox was carried on for 6 hours at 28° C. by adding one molar equivalent of hydrogen peroxide initially followed by the addition of another molar equivalent at each successive 2-hour interval. At the end of the reaction period, each oxidation mixture was suspended in water and extracted five times with equal volumes of chloroform. The residues from the combined chloroform extracts were studied as to chromatographic behavior, partition distribution, cholinesterase inhibition levels, formaldehyde yield and infrared spectra.

Other Methods.—For chromatographic studies silica gel was used with the solvent systems of Tsuyuki *et al.* (1955) except that 80:20 chloroform:*n*-hexane replaced the 20:80 chloroform:*n*-hexane reported. These columns were adequate to completely separate schradan from hexamethylphosphoramide, dimefox from schradan, and dimefox from trimethyl phosphorodiamidic fluoride. All metabolites from biological systems were chromatographed twice before obtaining infrared spectrograms on the products. Infrared analyses were made on a Baird spectrophotometer using NaCl cells with the metabolites as liquid films or as 10% solutions in reagent grade chloroform.

Results and Discussion.—*American Cockroach Distribution and Metabolism.*—The distribution and percentage hydrolysis of radioactive dimefox and schradan in certain organ systems of the American cockroach treated topically with sublethal doses are shown in table 1. In general the distribution patterns of the two phosphoramides were similar. Organs containing the highest concentration of radioactivity were the Malpighian tubules and hindgut. A different distribution pattern was indicated for these two radiolabeled materials by the hindgut accumulation as compared with a foregut accumulation reported for other organophosphates (Roan *et al.* 1950, Fernando *et al.* 1951, Acree *et al.* 1956). Schradan was much more biologically stable in all cockroach organs

Table 2.—Tissue distribution and stability of radioactive dimefox and schradan in male rats.

A. Distribution of radioactivity determined 5 to 6 minutes after subcutaneous treatment with acute lethal dose of dimefox (10γ/gm.). (Average of 2 replicates.)

Tissue	p.p.m. Based on Total Radioactivity	Per Cent Hydrolysis	Per Cent of Total Radioactivity in Tissue Residue
Brain	7.48	8	14
Blood	7.94	8	11
Heart	1.96	20	21
Intestine	5.38	4	15
Kidney	13.26	12	12
Liver	7.83	25	32
Muscle	2.56	8	36
Nerve Cord	6.79	0	9
Pancreas	6.95	6	14
Salivary Gland	4.62	9	35
Skin	4.22	2	—
Spleen	11.93	16	23
Stomach	1.68	10	5
Testis	3.70	11	9

B. Distribution of radioactivity 5 days after treatment with sublethal oral doses of dimefox and schradan (2.5 γ/gm.). (Average of 3 replicates of 2 rats each.)

Tissue	p.p.m. based on Total Radioactivity[a]	
	Dimefox	Schradan
Brain	0.18±0.03	0.31±0.08
Blood	0.15±0.06	0.27±0.11
Intestine	0.32±0.02	0.24±0.02
Liver	0.98±0.04	0.74±0.12
Nerve Cord	0.25±0.10	0.29±0.10

[a] Variation of results is indicated as standard errors.

examined than was dimefox. This stability difference was also observed in other cockroach metabolism experiments with the two amides.

Column chromatography of the chloroform extracts of dimefox-treated cockroaches yielded a major fraction of radioactive material which was less polar and exhibited greater anticholinesterase activity than unmetabolized dimefox and yielded formaldehyde on treatment with sulfuric acid (table 5). Both dimefox and schradan are relatively poor inhibitors of cholinesterase (Aldridge & Barnes 1952, Casida *et al.* 1953). It is difficult to compare the anticholinesterase activity of the dimefox metabolite with that reported for the schradan metabolite (Casida & Stahmann 1953, Heath *et al.* 1955, Spencer & O'Brien 1956) as the cholinesterase enzyme sources were different. Too little of the dimefox metabolites were recovered in the β80-20 (80% chloroform-20%*n*-hexane) or chloroform chromatographic fractions for anticholinesterase and partition coefficient determinations. With schradan-treated cockroaches a more polar metabolite was recovered from the β80-20 column fraction (table 5).

Based on formaldehyde, the cockroach α80-20 metabolite of dimefox (table 5) partitioned entirely into chloroform from an aqueous medium; whereas, the partition coefficient based on radioactivity was 38. This suggested that the formaldehyde-yielding material was eluted from

Table 3.—Relative excretion rates and stability of radioactive dimefox and schradan from male rats treated orally with 2.5 γ/gm.[a]

Hours After Treatment	Cumulative Per Cent of Total Radioactivity Excreted in Urine		Per Cent Hydrolysis	
	Dimefox	Schradan	Dimefox	Schradan
0– 2	7.4	6.6	73	53
2– 4	12.7	12.6	83	55
4– 8	15.9	16.5	86	62
8– 16	20.1	20.8	89	64
16– 24	22.5	22.8	89	66
24– 36	23.1	23.3	85	69
36– 48	23.5	23.6	86	84
48– 60	23.7	24.1	100	100
60– 72	23.9	24.2	100	100
72– 96	24.0	24.3	100	100
96–100	24.1	24.3	100	100

[a] Average of three replicates of two rats each.

silica gel in the same fraction with unmetabolized dimefox. The formaldehyde-yielding metabolite was distinctly different from paraformaldehyde which has a chloroform/water partition coefficient of 0.34 and formaldehyde with an 0.058 partition coefficient.

Infrared absorption spectra of the dimefox and schradan cockroach α80-20 metabolites compared with the unmetabolized materials showed decreased heights of peaks at 2.94 and 6.18μ and the occurrence of new peaks at 5.90 and 7.25μ (table 6). These spectral changes have also been noted in the rat liver metabolite of schradan (Casida *et al.* 1954) and in permanganate-oxidized schradan (Tsuyuki *et al.* 1955, Spencer & O'Brien 1956).

Mammalian Distribution and Metabolism.—Results of tissue distribution studies in rats treated subcutaneously with lethal doses of radioactive dimefox (table 2a) illustrate the rapidity with which the radioactivity was distributed to all parts of the body. The largest concentration of radioactivity was in the kidney and spleen followed by the liver, blood and brain. Even with such a short metabolism period, considerable hydrolysis of dimefox occurred with possible incorporation of the hydrolytic materials into other products of normal metabolism as evidenced by the large amount of non-water soluble radioactivity remaining in the tissue residue. The radioactivity

Table 4.—Distribution and stability in plants of radioactive dimefox and hexamethylphosphoramide. (Average of 4 replicates.)

Dimefox Treated Pea Plants	p.p.m. Based on Total Radioactivity			Per Cent Hydrolysis		
	Days After Treatment			Days After Treatment		
	1	3	5	1	3	5
Roots	1536	67	64	0	0	0
Stems	1611	283	161	1	18	21
Leaves	2316	1720	1681	10	23	28
Hexamethylphosphoramide Treated Bean Plants	1	3	7	12	1	3	7	12
Roots	915	47	27	15	—	—	—	—
Stems	361	116	81	72	—	—	—	—
Leaves	892	2075	1928	1752	4	6	7	12

Table 5.—Chromatographic behavior, partition distribution, and anticholinesterase activity of certain phosphoramides and their degradation products.

Phosphoramide or Derivative	Per Cent Phosphorus				Part. Coeff. $HCCl_3/H_2O$			HCHO on Degradation[a]	M. Concentration for 50% ChE Inhibition
	α80-20	β80-20	$HCCl_3$	MeOH	α80-20	β80-20	$HCCl_3$	α80-20	α80-20
Dimefox	100	0.0	0.0	0.0	16	—[b]	—	A	1.0×10^{-2}
Dimefox: Peracetic	94.0	3.6	0.3	2.2	11	4.7	1.2	P	1.0×10^{-6}[c]
Dimefox: Permanganate	96.5	2.3	0.2	1.1	9.2	3.0	1.2	A	1.0×10^{-2}
Dimefox: Peroxide	93.1	5.1	0.6	1.3	18	7.0	6.4	A	1.0×10^{-2}
Dimefox: Cockroach	94.3	0.7	0.5	4.6	37	—	—	P	1.3×10^{-5}[c]
Dimefox: Rat liver (*in vivo*)	95.9	2.7	<0.01	1.4	17	1.7	—	—	5.0×10^{-5}
Dimefox: Rat liver (*in vitro*)	95.0	3.2	<0.01	1.8	42	—	—	P	2.0×10^{-5}[c]
Dimefox: Pea plant	93.2	3.3	1.1	2.4	25	—	—	P	—
Trimethylphosphorodiamidic fluoride	0.0	100	0.0	0.0	—	1.1	—	A	6.7×10^{-6}
Schradan	100	0.0	0.0	0.0	7.1	—	—	A	—
Schradan: Cockroach	91.9	6.8	0.0	1.3	7.7	2.0	—	P	—
	α$HCCl_3$	β$HCCl_3$		MeOH	α$HCCl_3$	β$HCCl_3$		α$HCCl_3$	
Hexamethylphosphoramide	100	0.0		0.0	6.6	—		A	—
Hexamethylphosphoramide: Rat liver (*in vitro*)	92.8	4.2		3.1	9.0	5.6		P	—
Hexamethylphosphoramide: Bean plant	90.0	6.7		3.3	8.5	2.9		P	—

[a] P = organically bound formaldehyde present; A = formaldehyde absent.
[b] Insufficient amounts for determinations.
[c] Molar concentration of inhibitor based on formaldehyde assuming one mole formaldehyde liberated on degradation per mole of inhibitor.

in various tissues 5 days after treatment was about the same for rats treated orally with sub-lethal doses of dimefox and schradan, the highest concentration of radioactivity being in the liver (table 2b).

Both dimefox and schradan metabolites were rapidly excreted in the urine of rats and the relative rates of excretion were almost identical (table 3). About 24% of the administered radioactivity from each compound was excreted in the urine within 5 days. Initially dimefox was excreted in the urine more in an ionized form than schradan, but both radiolabeled materials were excreted as completely water-soluble metabolites by the end of 2 days after treatment. The total amount of radioactivity excreted in a given period of time was largely independent of the total urine production for the same time interval; consequently, the relative excretion rates were calculated as cumulative per cent of total administered radioactivity rather than of radioactivity per ml. of urine. About 15% of the radioactivity from either amide was eliminated in the feces within 5 days following oral administration.

In vivo and in vitro Rat Liver Metabolism.—Activation of dimefox by rat liver to a more potent anticholinesterase agent was demonstrated both *in vivo* and *in vitro*. The liver metabolites in the α80-20 fraction partitioned more into chloroform from an aqueous medium than unmetabolized dimefox, yielded formaldehyde on treatment with sulfuric acid and contained a more active anticholinesterase agent (table 5). A more polar liver metabolite was recovered in the β80-20 fraction from *in vivo* metabolism. The conversion of dimefox by rat liver slices to an unstable more active cholinesterase inhibitor has previously been demonstrated (Aldridge & Barnes 1952, Fenwick *et al.* 1957). However, Okinaka *et al.* (1954) in similar studies observed no activation but only detoxification of dimefox by rat liver slices.

In vitro metabolism of hexamethylphosphoramide (table 5, chloroform fraction) and schradan by rat liver slices also produced materials that yielded formaldehyde and were more chloroform-soluble than the parent compounds. Infrared spectrograms of these metabolites showed the general spectral changes as noted for the dimefox cockroach metabolite (table 6).

Plant Distribution and Metabolism.—Radiolabeled dimefox and hexamethylphosphoramide were rapidly absorbed through the roots and translocated to the leaves of pea and bean plants, respectively (table 4). Dimefox in in this experiment was selectively absorbed from the nutrient solution as has been previously reported by David (1952). The loss of over one-half of the radioactive dimefox from plant tissues within 3 days from the time the insecticide source was removed might be partially accounted for on the basis of volatilization. Hexamethylphosphoramide was less volatile and more stable in plants than dimefox. Dimefox has previously been noted to be more volatile from plants than schradan (David 1952, David & Gardiner 1951). Studies to determine the relative volatility of radioactive dimefox and schradan from glass planchets at room temperature showed that dimefox was 56% volatilized at the end of 1 hour while schradan was only 8% volatilized, the values at the end of 24 hours were 88% and 33%, respectively. Despite its volatility, the stability of dimefox in plants may account for its prolonged control of certain plant feeding insects when applied as a systemic under field conditions (David 1952, Hanna *et al.* 1952, Bond 1953). Dimefox and hexamethylphosphoramide at 2000 p.p.m. in the nutrient resulted in severe necrosis of the plant foliage, the greatest injury occurring with dimefox.

Inspection of the infrared spectrograms of the dimefox pea plant metabolite and schradan and hexamethylphosphoramide bean plant metabolites indicated that the three amides were metabolized in a similar manner (table

Table 6.—Changes in infrared absorption spectra of phosphoramides after oxidation[a] (10% chloroform solution: location of broad peaks indicated to the closest 0.05 microns).

PHOSPHORAMIDE OR DERIVATIVE	COLUMN FRACTION	NEW PEAKS	LOST PEAKS	DECREASED HEIGHT
Dimefox: Peracetic[b]	α80-20	none	none	none
Dimefox: Peracetic[b]	β80-20	5.95; 7.27	none	2.94; 6.18
Dimefox: Peracetic	$HCCl_3$	5.90; 7.27; 9.00	6.18; 6.72	2.94
Dimefox: Permanganate[b]	α80-20	5.90	6.18	2.94
Dimefox: Permanganate[b]	β80-20	5.95; 7.06; 7.27	6.18; 8.50; 11.80	2.94; 8.10
Dimefox: Permanganate	$HCCl_3$	3.12; 6.00; 7.20; 9.08	6.18	none
Dimefox: Peroxide[b]	α80-20	none	none	none
Dimefox: Peroxide[b]	β80-20	5.90; 7.27	8.10; 11.80	2.94; 6.18
Dimefox: Peroxide	$HCCl_3$	5.90; 7.27	none	2.94; 6.18; 8.10; 11.80
Dimefox: Cockroach	α80-20	5.90; 7.25	none	2.94; 6.18
Dimefox: Pea Plant	α80-20	5.90	none	2.94; 6.18
Trimethylphosphorodiamidic fluoride[bc]	β80-20	3.05; 7.18; 9.05	none	9.33
Schradan: Rat liver (*in vitro*)	$\beta HCCl_3$	5.90	none	2.94; 6.18
Schradan: Bean plant	$\beta HCCl_3$	5.90	none	2.94; 6.18
Schradan: Cockroach	α80-20	5.90; 7.25	6.18	2.94
Hexamethylphosphoramide: Rat liver (*in vitro*)	$\alpha HCCl_3$	5.90	none	6.18
Hexamethylphosphoramide: Bean plant	$\alpha HCCl_3$	5.90	none	6.18

[a] Infrared spectra on all biological metabolites were compared with identical biological fractions in which the phosphoramide or metabolites were not present to correct for peak changes due to biological impurities.
[b] Spectra for these fractions also obtained with liquid films and all the above changes were substantiated.
[c] Known compound obtained from Dr. G. Schrader, Farbenfabriken Bayer, and purified chromatographically.

6). The appearance of a new peak at 5.9μ and decreased height of the peak at 6.2 μ were characteristic for all three materials. Further evidence that the three amides undergo similar metabolic changes in plants was obtained from their increased chloroform solubility from an aqueous medium and the formation of a group which yielded formaldehyde on treatment with acid.

Chemical Oxidation of Dimefox.—Dimefox which had been oxidized with peracetic acid, potassium permanganate or hydrogen peroxide yielded four fractions on chromatography. These fractions were compared with the parent compound and the biological metabolites in respect to partitioning properties, anticholinesterase activity, formaldehyde formation on degradation (table 5) and infrared spectra (table 6). The reaction products contained about 5% hydrolyzed materials in all instances and these were removed with chloroform/water partitioning before chromatography.

Each successive chromatographic fraction contained derivatives which were progressively more polar in the order of elution. The products from peroxide oxidation of dimefox were distinctly less polar than the corresponding column eluate fractions from peracetic or permanganate oxidation of dimefox (table 5). Solubility differences for the products from peracetic acid and sodium hypochlorite oxidation of schradan have been reported (Tsuyuki *et al.* 1955). Trimethylphosphorodiamidic fluoride, one of the possible products of dimefox degradation, yielded a partition ratio based on phosphorus similar to the chloroform-eluate fractions from permanganate and peracetic acid oxidation of dimefox. However, the infrared spectra of these two chloroform-eluate fractions were not identical to known trimethylphosphorodiamidic fluoride (table 6). These results indicate that evidence for the characterization of phosphoramide degradation products based on change in solubility of phosphorus (Heath *et al.* 1955) is indicative but not sufficiently conclusive to establish the degradative mechanism involved.

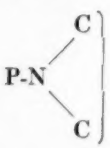
Peracetic acid oxidized dimefox yielded formaldehyde on degradation in all three column fractions. The partition coefficients (chloroform/water) for the fractions of the oxidized phosphoramides were similar either when based on formaldehyde or on phosphorus and the phosphorus and formaldehyde from these α80-20 and β80-20 fractions co-chromatographed indicating that the formaldehyde-yielding group in the fractions was a part of the organophosphorus molecule. Although the α80-20 eluate fraction of peracetic acid-oxidized dimefox was found to be identical to the dimefox infrared spectra (table 6), it contained from 0.1 to 0.2% of a formaldehyde-yielding material which was a very active anticholinesterase agent (table 5). The β80-20 eluate fraction contained a more polar and even more potent anticholinesterase agent. The chloroform fraction contained mostly formaldehyde not associated with a phosphorus molecule. Products from peroxide and permanganate oxidation of dimefox did not yield formaldehyde under the conditions employed in these experiments. In a similar manner to schradan (Casida *et al.* 1953, Tsuyuki *et al.* 1955, Spencer & O'Brien 1956), dimefox was oxidized to several potent anticholinesterase agents among which were formaldehyde-yielding phosphoramides both less polar (α80-20) and more polar (β80-20) than the original phosphoramide.

No two infrared spectra for the chemical oxidation products were identical except for the fractions containing unreacted dimefox (table 6). The spectra probably represent mixtures of degradation products from the various degrees of attack on the phosphoramide molecule. The infrared spectra of the eluate fractions from chemically oxidized dimefox showed definite similarities to each other and to the metabolites of the three phosphoramides from biological systems: *i.e.* decreased height of the peak at 2.9μ, loss or decreased height of the peak at 6.2μ and the appearance of new peaks at about 5.9 and 7.2μ. Functional groups associated with these spectral changes have not been definitely established for this class of compounds. However, the absorption band at about 5.9μ is present in many oxidized phosphoramides. An infrared spectra of a

purified metabolite of schradan from rat liver slices contained a new peak at 5.9μ (Casida *et al.* 1954). Later, Tsuyuki *et al.* (1955) showed the same absorption band in peracetic acid and sodium hypochlorite-oxidized schradan and attributed its presence to an N→O bond based on the presence of a similar band in trimethylamine oxide dihydrate which was absent in trimethylamine. Randall *et al.* (1949) reported the presence of this band in triethylamine oxide and triethylamine oxide dihydrate and its absence in diethylamine and triethanolamine. Loss of the 5.9μ absorption band without further alteration of the infrared spectra in permanganate oxidized-schradan has been reported to result from hydrolysis at pH 10 and 45° C. (Spencer & O'Brien 1956). These workers further indicated that this band was not present in two of the suspected degradation products of schradan, heptamethylpyrophosphoramide and heptamethylpyrophosphoramide N-methoxide. Formaldehyde content of oxidized phosphoramides was not necessarily correlated with the 5.9μ peak (tables 5 & 6). In this study the decrease in height of the 6.2μ band was related to increased intensity of the 5.9μ band and these absorption bands were more prominent when the materials were run as liquid films rather than as chloroform solutions.

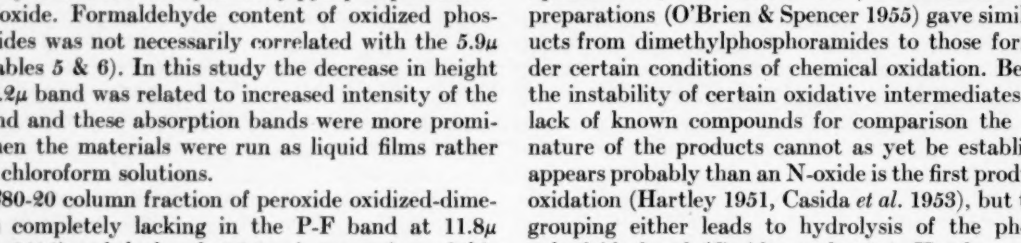
The β80-20 column fraction of peroxide oxidized-dimefox was completely lacking in the P-F band at 11.8μ (Larsson 1952) and the band at 8.1μ. A comparison of this spectra with the rat liver and bean metabolites of hexamethylphosphoramide showed them to be identical. Very similar changes were also noted for the β80-20 fraction of permanganate oxidized-dimefox. The possibility that these chromatographic fractions (β80-20 fraction, peroxide and permanganate oxidized-dimefox) were merely dimefox contaminants was ruled out on the basis of chromatographic behavior and infrared analysis of known dimefox. The complex reactions which could yield these results are not understood.

The chloroform fraction of permanganate oxidized-dimefox showed many similarities in infrared spectra to trimethylphosphorodiamidic fluoride including a 3.1μ band associated with N-H stretching (Holmstedt & Larsson 1951) indicating that this fraction might contain trimethylphosphorodiamidic fluoride or other methylphosphorodiamidic fluorides. Absorption bands corresponding to the P-NH stretching vibrations were not present in any other fraction of the oxidized materials. Likewise, the P-N absorption band (in the skeleton



at 13.2μ (Larsson 1952) was not decreased in intensity in spectra of the column eluates determined as a liquid film other than this chloroform fraction of permanganate oxidized-dimefox (table 6) indicating that the CH_3 groups were not replaced by H groups in other fractions (Holmstedt & Larsson 1951). None of the column fractions from dimefox oxidation mixtures eluted with chloroform or *n*-hexane-chloroform showed absorption bands in the region at 2.8 to 3.1μ similar to those usually associated with OH groups in carbon compounds.

The oxidative mechanisms of phosphoramides although frequently investigated are poorly understood. *In vivo* metabolism in insects (Casida *et al.* 1953, 1954, O'Brien & Spencer 1955, Tsuyuki *et al.* 1955), mammals (Casida *et al.* 1953, 1954) and *in vitro* metabolism in liver slices (Casida *et al.* 1953, 1954, Tsuyuki *et al.* 1955, O'Brien & Spencer 1955, Fenwick *et al.* 1957) and cockroach intestine preparations (O'Brien & Spencer 1955) gave similar products from dimethylphosphoramides to those formed under certain conditions of chemical oxidation. Because of the instability of certain oxidative intermediates and the lack of known compounds for comparison the isomeric nature of the products cannot as yet be established. It appears probably than an N-oxide is the first product from oxidation (Hartley 1951, Casida *et al.* 1953), but that this grouping either leads to hydrolysis of the phosphoric anhydride bond (Casida *et al.* 1954, Heath *et al.* 1955) or isomerizes to a more stable derivative which then yields a monomethylphosphoramide (Tsuyuki *et al.* 1955, Spencer & O'Brien 1956). Among the possible metabolic intermediates are:



I II III IV V

and certain cyclic oxides of the phosphoramide grouping. Certain products from chemical oxidation of phosphoramides yield formaldehyde on treatment with acid (Casida *et al.* 1952, Casida & Stahmann 1953, Tsuyuki *et al.* 1955, O'Brien & Spencer 1955, Spencer & O'Brien 1956) and certain of the products are associated with an infrared absorption band at 5.9μ (Casida *et al.* 1954, Tsuyuki *et al.* 1955, Spencer & O'Brien 1956). One derivative from oxidation of phosphoramides is more and another less polar than the original phosphoramide. The antiesterase activity of the oxidative intermediates is more closely associated with the groupings that yield formaldehyde on treatment with acid than with the 5.9μ absorption band. Except for the greater instability of dimefox and its derivatives, the metabolic intermediates appear to be similar to those of schradan. These results based on infrared spectra, chromatographic characteristics and formaldehyde determination agree with the conclusions of Fenwick *et al.* (1957) based on anticholinesterase measurements. Further chemical work is in progress in an attempt to resolve this problem.

SUMMARY.—Comparative studies were made with dime-

fox, schradan and hexamethylphosphoramide on their chemical oxidation and metabolism in insects, mammals and plants. Dimefox and schradan were distributed in a similar manner among the organ systems of white rats, and both accumulated in the hindgut of American cockroaches (*Periplaneta americana* (L.)). These two phosphoramides were excreted at identical rates in the urine and feces of rats. In both the cockroaches and rats, scradan was more stable to hydrolysis than dimefox. Dimefox was more readily absorbed by plants than hexamethylphosphoramide, and was more volatile from but less stable within the plant tissues.

The biological and chemical oxidation products of dimefox were compared with those of schradan and hexamethylphosphoramide. Within the insects, plants and mammals investigated, each of these compounds was metabolized to form oxidized derivatives which decomposed on treatment with acid to yield formaldehyde. The non-hydrolytic biological metabolites of dimefox, schradan and hexamethylphosphoramide contained a new infra-red absorption peak at 5.90 microns and a decreased height of absorption peaks at 2.94 and 6.18 microns. These changes in the infra-red spectra also occurred on oxidation of dimefox with peracetic acid, potassium permanganate and hydrogen peroxide. Dimefox and schradan were converted to more active anticholinesterase agents within insects, mammals and plants. The anticholinesterase activity of the oxidative intermediates was more closely associated with the groupings that yielded formaldehyde on treatment with acid than with the groupings responsible for the 5.90 micron absorption band. With dimefox, schradan and hexamethylphosphoramide, one oxidative derivative was more and another less polar than the original phosphoramide. Because of the instability of certain oxidative intermediates and the lack of known compounds for comparison, the isomeric configuration of the derivatives cannot as yet be established. Except for the greater instability of dimefox and its derivatives, the metabolic intermediates appeared to be similar to those of schradan and hexamethylphosphoramide.

REFERENCES CITED

Acree, F., Jr., F. H. Babers, and N. Mitlin. 1956. Preparation of P-32 labeled Bayer L 13/59 and DDVP, and their absorption and translocation in the American cockroach. Jour. Econ. Ent. **49:** 808–12.

Adkins, T. R., W. L. Sowell, and F. S. Arant. 1955. Systemic effect of selected chemicals on the bed bug and lone star tick when administered to rabbits. Jour. Econ. Ent. **48:** 139–41.

Aldridge, W. N., and J. M. Barnes. 1952. Some problems in assessing the toxicity of the organophosphorus insecticides toward mammals. Nature **169:** 345–7.

Allen, R. J. L. 1940. The estimation of phosphorus. Biochem. Jour. **34:** 858–65.

Arthur, B. W. and J. E. Casida. 1957. Metabolism and selectivity of 0,0-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate and its acetyl and vinyl derivatives. Jour. Agric. Food Chem. **5:** 186–92.

Bond, J. A. B. 1953. Trunk absorption of a systemic chemical by coffee. Bull. Ent. Res. **44:** 97–100.

Casida, J. E., T. C. Allen, and M. A. Stahmann. 1952. Reaction of certain octamethylpyrophosphoramide derivatives with chymotrypsin. Jour. Amer. Chem. Soc. **74:** 5548.

Casida, J. E., T. C. Allen, and M. A. Stahmann. 1953. Enzymatic and chemical oxidation of dimethylphosphoramides to biologically active dimethylphosphoramide oxides. Nature **172:** 243–45.

Casida, J. E., T. C. Allen, and M. A. Stahmann. 1954. Mammalian conversion of octamethylpyrophosphoramide to a toxic phosphoramide N-oxide. Jour. Biol. Chem. **210:** 607–16.

Casida, J. E., P. E. Gatterdam, L. W. Getzin, Jr., and R. K. Chapman. 1956. Residual properties of the systemic insecticide 0,0-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate. Jour. Agric. Food Chem. **4:** 236–43.

Casida, J. E. and M. A. Stahmann. 1953. Metabolism and mode of action of schradan. Jour. Agric. Food Chem. **1:** 883–8.

Comar, C. L. 1955. Radioisotopes in Biology and Agriculture. McGraw-Hill Book Co., Inc., N.Y.

Cook, H. G., J. D. Ilett, B. C. Saunders, G. J. Stacey, H. G. Watson, I. G. E. Wilding, and S. J. Woodcock. 1949. Esters containing phosphorus IX. Jour. Chem. Soc. **1949:** 2921–7.

David, W. A. L. 1952. Insecticidal-action studies with bisdimethylaminofluorophosphine oxide containing P-32 phosphorus. Ann. Appl. Biol. **39:** 203–10.

David, W. A. L., and B. O. C. Gardiner. 1951. Investigations on the systemic insecticidal action of sodium fluoroacetate and of three phosphorus compounds on *Aphis fabae* Scop. Ann. Appl. Biol. **38:** 91–110.

DuBois, K. P., J. Doull, and J. M. Coon. 1950. Studies on the toxicity and pharmacological action of octamethylpyrophosphoramide (OMPA; Pestox III). Jour. Pharmacol. Exptl. Therap. **99:** 376–93.

Fenwick, M. L., J. R. Barron, and W. A. Watson. 1957. The conversion of dimefox into an anticholinesterase by rat liver *in vitro*. Biochem. Jour. **65:** 58–67.

Fernando, H. E., C. C. Roan, and C. W. Kearns. 1951. The penetration, distribution and metabolism of organic phosphates in the American roach, *Periplaneta americana* (L.). Ann. Ent. Soc. America **44:** 551–65.

Forbes, M. C., C. A. Roswell, and R. N. Maxson. 1946. Phosphorus (III) trichloride. Inorganic synthesis. **2:** 145–7.

Hanna, A. D., W. Heatherington, and E. Judenko. 1952. Control of mealybug vectors of the swollen shoot virus by a systemic insecticide. Nature **169:** 334–5.

Hanna, A. D., E. Judenko, and W. Heatherington. 1956. The control of *Crematogaster* ants as a means of controlling the mealybugs transmitting the swollen-shoot virus disease of cacao in the Gold Coast. Bull. Ent. Res. **47:** 219–26.

Hartley, G. S. 1951. First steps in the biochemistry of systemic insecticides. Section 13, Pesticides, 15th International Congress of Pure and Applied Chemistry. N.Y., September 1951.

Heath, D. F., and P. Casapieri. 1951. Hydrolysis of dimethylamides of phosphoric acids. Trans. Fara. Soc. **47:** 1093–1101.

Heath, D. F., D. W. J. Lane, and M. Llewellyn. 1952. Studies on commercial octamethylpyrophosphoramide. IV. The decomposition of pyrophosphoric acid tetra (dimethylamide) and orthophosphoric acid tri(dimethylamide) in the living plant. Jour. Sci. Food and Agric. **2:** 69–73.

Heath. D. F., D. W. J. Lane, and P. O. Park. 1955. The decomposition of some organophosphorus insecticides and related compounds in plants. Phil. Trans., Series B. **239:** 191–214.

Holmstedt, B., and L. Larsson. 1951. Infra-red absorption

spectra of some organic phosphoryl compounds. Acta Chem. Scand. 5: 1179–86.

Krueger, H. R., and J. E. Casida. 1957. Toxicity of fifteen organophosphorus insecticides to several insect species and to rats. Jour. Econ. Ent. 50: 356–8.

Krueger, R. 1949. Über die quantitative Bestimmung Kleiner Mengen Glykokoll in Blut, Harn, and protein hydrolsaten. Helvetica Chim. Acta. 32: 238–51.

Larsson, L. 1952. A spectrophotometric study in infra-red of the hydrolysis of dimethylamido-ethoxy-phosphoryl cyanide (Tabun). Acta Chem. Scand. 6: 1470–6.

Michelbacher, A. E., and E. Oatman. 1955. Marked suppressing action of schradan on the walnut aphid. Jour. Econ. Ent. 48: 768–9.

O'Brien, R. D., and E. Y. Spencer. 1953. Metabolism of octamethylpyrophosphoramide by insects. Jour. Agric. Food Chem. 1: 946–50.

O'Brien, R. D., and E. Y. Spencer. 1955. Further studies on the insect metabolism of octamethylpyrophosphoramide. Jour. Agric. Food Chem. 3: 56–61.

Okinaka, A. J., J. Doull, J. M. Coon, and K. P. DuBois. 1954. Studies on the toxicity and pharmacological actions of bis(dimethylamido) fluorophosphate (BFP). Jour. Pharmacol. Exptl. Therap. 112: 231–45.

Pound, W. 1953. Brit. Pat. No. 688,760. March 11, 1953.

Randall, H. M., R. G. Fowler, N. Fuson, and J. R. Dangl. 1949. Infrared Determination of Organic Structures. Van Nostrand, N.Y.

Ripper, W. E., R. M. Greenslade, and G. S. Hartley. 1950. A new systemic insecticide bis(bis-dimethylamino) phosphonous anhydride. Bull. Ent. Res. 40: 481–501.

Roan, C. C., H. E. Fernando, and C. W. Kearns. 1950. A radiobiological study of four organic phosphates. Jour. Econ. Ent. 43: 319–25.

Schrader, G. 1947. The development of new insecticides. B.I.O.S. Final Report No. 714 (revised). London: H.M.S.O.

Spencer, E. Y., and R. D. O'Brien. 1956. Permanganate oxidation products of schradan. Jour. Agric. Food Chem. 5: 123–27.

Tsuyuki, H., M. A. Stahmann, and J. E. Casida. 1955. Preparation, purification, isomerization and biological properties of octamethylpyrophosphoramide-N-oxide. Jour. Agric. Food Chem. 3: 922–32.

Vanscheidt, A. A., and V. M. Tolstopyatov. 1920. The preparation of phosphoryl chloride by the simultaneous action of water and chlorine on phosphorus trichloride. Jour. Russ. Phys.-Chem. Soc. 52: 270–84.

Resistance of *Culex tarsalis* Coq. to DDT in Oregon[1]

GAINES W. EDDY, THEODORE L. HOPKINS, and WILLIAM E. ROBBINS, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

The mosquito, *Culex tarsalis* Coq., is perhaps the most important arthropod involved in the transmission and maintenance of the etiological agents of the viral encephalitides affecting man and animals (Dean 1955, Hammon & Reeves 1942, Lennettee 1955). It is distributed over most of the United States, except for the northeastern part, and over a wide portion of southwestern Canada (Carpenter & LaCasse 1955, Stage *et al.* 1952). Although the mosquito breeds in many types of situations, it is a heavy breeder in grassland areas (Lindquist 1955, Rapp 1955). It is especially numerous in irrigated pastures (Husbands & Rosay 1953, Lindquist 1955), and in such places is an important pest of livestock. Control of the species therefore becomes a concern to all.

In California, where *tarsalis* is very important from both disease and pest standpoints, control with DDT presented no problem until about 1949. Indicated resistance to DDT was first reported in that year (Smith 1949) and definitely established in 1951, at which time larvae from treated areas were shown to be about 10 times as resistant as larvae from untreated areas (Gjullin & Peters 1952). Resistance to DDT forced mosquito-control agencies in some areas to switch to other insecticides in 1952, and by 1955 its use for mosquito control in California had been all but abandoned (Stivers 1956). During the last 2½ years malathion has been substituted for DDT in some areas, with the result that *tarsalis* has developed considerable resistance to this material (Gjullin & Isaak 1957).

Although DDT has been the principal insecticide used in mosquito control in Oregon for several years, no resistance problems have developed there so far as the authors are aware. In Multnomah County and adjoining areas where control operations have been rather extensive for a number of years, resistance to DDT might well have developed had the control activities been concerned more with *tarsalis* than with species of floodwater *Aedes*.

The most extensive and sustained program which involved the control of *tarsalis* was initiated in Lane County in 1953. In this county, as well as in the Willamette Valley generally, log ponds produce tremendous numbers of *tarsalis* and other mosquitoes. DDT was used effectively through the 1955 season except in one log pond located at Oakridge (Buehler 1955). In this particular pond poor control of *tarsalis* was noted near the end of the season and again during the summer of 1956 by the director of the Lane County mosquito control program (Buehler 1956). Bioassays of the larvae by the authors indicated a very high degree of resistance. These experiments, along with the establishment of a DDT-resistant colony, are reported herein.

ESTABLISHMENT AND MAINTENANCE OF COLONIES.—A *tarsalis* colony nonresistant to DDT was available for comparative test purposes. The colony was originally obtained from workers of the National Institutes of Health's Spotted Fever Laboratory, and the rearing methods used were adapted from those described by them (Brennan & Harwood 1953, Hubert *et al.* 1954). The larvae were reared in white enamel pans approximately 13¼ inches in diameter and 4¼ inches deep, containing 3 liters of tap water. Hi-Protein Supplement (product of Misco Mills, Missoula, Mont.) was used as the larval food. The rearing room was maintained at 78 to 80° F. The adults were kept in an adjoining room 7×9×10 feet in which the temperature and relative humidity were maintained at 75° F. and 70%, respectively. The females were allowed to feed on chickens twice weekly, and the males were given sugar

[1] Accepted for publication July 26, 1957.

water. The room had a window space of 27×48 inches. The sleeve-equipped screen rearing cages ranged in size from approximately 16×16×16 to 20×36×36 inches. No attempt was made to control the period or amount of light.

After preliminary experiments conducted in July 1956 had established that *tarsalis* larvae from Oakridge were resistant to DDT, several thousand were obtained for colonization. In the light of experiences of others (Brennan & Harwood 1953, Hubert *et al.* 1954, Lungstrom 1954), considerable trouble was expected in rearing this "wild strain." However, they were subjected to the same procedures as the nonresistant regular colony. After approximately 11 generations the colony is thriving, and large numbers are being produced for experimental purposes.

TESTS WITH FOURTH-INSTAR LARVAE.—The technique employed was similar to that used by workers in the Department of Agriculture for a number of years (King 1954). The desired amount of DDT in acetone was added to 250 ml. of distilled water contained in 400-ml. beakers. The amount of acetone was not greater than 0.2 nor less than 0.04% of the water volume. Twenty fourth-instar larvae were used per test, and mortality was recorded after 24 hours. The tests were conducted at 78 to 80° F.

Two series of tests were run, one with first-generation larvae, and another a later, about the 11th ±3, generation. An accurate record of the number of generations was not kept, but from the time required for completing the life cycle it could not have been more than 14, and was believed to be no less than 8.

The results obtained against fourth-instar larvae are shown in table 1. A comparison of the data reveals the extreme resistance in the Oakridge strain. The LC-50 of the nonresistant colony was 0.003 p.p.m., whereas 50 p.p.m. caused only 82% mortality of first-generation larvae. This high concentration was also required to cause complete kill of the 11th generation. Although an accurate measurement of resistance between the Oakridge colony and the nonresistant laboratory colony is not easily ascertained, on the basis of the LC-50 the Oakridge colony would be at least 1000 times more resistant, and on the basis of the LC-90 would be definitely more than 5000 times. Other tests indicated that no more than a two-fold difference existed between *tarsalis* larvae from untreated log ponds and those of the laboratory nonresistant colony.

Table 1.—Tests with DDT against fourth-instar larvae of the Oakridge strain of *Culex tarsalis*.[a]

P.P.M.	PER CENT MORTALITY AFTER 24 HOURS	
	First Generation	Eleventh Generation
0.5	0	11
1	6	30
2.5	0[b]	41
5	57	64
10	62	84
15	35[b]	91
20	83	—
25	—	91
30	82	—
35	—	96
40	76	—
50	82	100
Controls	1	0
Laboratory nonresistant colony	LC-50: 0.003	LC-90: 0.01

[a] Four or five tests of 20 larvae each.
[b] Single test

Table 2.—Tests with DDT against newly hatched *Culex tarsalis* larvae of the 11th (±3) generation of the Oakridge colony.[a]

P.P.M.	PER CENT MORTALITY AFTER HOURS INDICATED		
	24	48	72
0.2	100 (2)		
.1	70 (6)	79 (4)	90 (2)
.075	20 (2)	48 (2)	48 (2)
.05	24 (6)	38 (6)	33 (2)
.025	4 (2)	6 (2)	—
Controls	1 (5)	5 (6)	9 (3)

[a] 50 or 100 larvae per test. Number of tests given in parenthesis.

The data obtained in these tests probably represent only the approximate resistance of the larvae, owing to the poor solubility of DDT in water. The true solubility of DDT in water is 0.0002 p.p.m. although it can form colloidal solutions up to 0.2 p.p.m. (Brown 1951). Since the rates used in these tests are all above this, the mortalities may have been higher than justified. In fact, it is suspected that at the higher concentrations the material precipitating on the surface of the water may have contributed to the mortality of the larvae by mechanically interfering with their activity and/or breathing.

TESTS WITH NEWLY HATCHED LARVAE.—Tests were also conducted with newly hatched larvae. They were made in connection with obtaining data for use in selection rather than establishing resistance. White enamel pans approximately 7¾ inches in diameter and 3¼ inches deep were used as test containers. DDT in acetone was added to 1 liter of distilled water in the pans. The concentration of DDT in the acetone was adjusted so that the amount of acetone put in the water was not greater than 0.02 nor less than 0.01%. Fifty or 100 larvae were then placed in each pan, followed by approximately 35 mg. of larval food (Hi-Protein Supplement). The same amount was also added after 24 and 48 hours where observations were made for such periods.

In preliminary experiments conducted with DDT against newly hatched larvae of the Oakridge colony, from 50 to 90% of the larvae were killed when they were reared in water containing 0.5 p.p.m. of DDT. No further tests were made until the 11th generation (table 2), at which time 0.2 p.p.m. caused 100% mortality in 24 hours and 0.075 p.p.m. caused 48% in 72 hours. Observations on pupation and emergence indicated that little mortality occurred beyond 72 hours.

The data on newly hatched and fourth-instar larvae cannot be directly compared owing to the wide differences in test methods.

DISCUSSION.—As can be seen from table 1, there was a definite loss of resistance between the first and eleventh generations. Loss of resistance appeared to be greater in the newly hatched than in the fourth-instar larvae. The

colony has been divided into two groups, one of which is subjected to DDT (0.075 p.p.m.) in an attempt to increase resistance, and the other is being retained to study loss of resistance.

The resistance of *tarsalis* to DDT reported above is believed to represent the first instance of insecticide resistance in mosquitoes in Oregon. Malathion was used successfully near the close of the 1956 mosquito season to control these mosquitoes (Buehler 1956). Although laboratory tests made by the authors indicated no resistance to malathion, it would be expected on continued use in view of research findings in California (Gjullin & Isaak 1957).

SUMMARY.—Larvae of the mosquito, *Culex tarsalis* Coq., collected from a log pond at Oakridge, Oregon, were found to be resistant to DDT. They were shown to be at least 1000 times more resistant than a laboratory nonresistant colony on the basis of the LC-50, and more than 5000 times on the basis of LC-90. For instance, the LC-50 of the nonresistant colony was 0.003 p.p.m., whereas 50 p.p.m. caused only 82% mortality of the resistant larvae. Some loss of resistance was noted in tests made after the 11th generation. The colonization and maintenance of the colonies are discussed.

REFERENCES CITED

Brennan, James M., and Robert F. Harwood. 1953. A preliminary report on the laboratory colonization of the mosquito, *Culex tarsalis* Coquillett. Mosquito News **13**(2): 153–7.

Brown, A. W. A. 1951. Insect control by chemicals. 817 pp. John Wiley & Sons.

Buehler, M. H. 1955. Third annual report Lane County mosquito control program. Lane County Health Dept., Eugene, Oreg.

Buehler, M. H. 1956. Fourth annual report Lane County Health Dept., Eugene, Oreg.

Carpenter, Stanley J., and Walter J. LaCasse. 1955. Mosquitoes of North America (North of Mexico). 361 pp. Univ. Calif. Press.

Dean, Ben H. 1955. Veterinary aspects of arthropod-borne encephalitides. Proc. California Mosquito Control Assoc. and Amer. Mosquito Control Assoc. Conf., pp. 41–3.

Gjullin, C. M., and Lewis W. Isaak. 1957. Present status of mosquito resistance to insecticides in the San Joaquin Valley of California. Mosquito News **17**(2): 67–70.

Gjullin, C. M., and Richard F. Peters. 1952. Recent studies of mosqutio resistance to insecticides in California. Mosquito News **12**(1): 1–7.

Hammon, W. McD., and W. C. Reeves. 1942. *Culex tarsalis* Coq. a proved vector of St. Louis encephalitis Proc. Soc. Exptl. Biol. and Med. **51**: 142–3.

Hubert, Alexander A., William A. Rush, and James M. Brennan. 1954. Simplified techniques for the continuous rearing of *Culex tarsalis* with additional notes and observations. Mosquito News **14**(2): 75–8.

Husbands, Richard C., and Bettina Rosay. 1953. Irrigated pasture mosquito ecology studies—1952. Proc. California Mosquito Control Assoc., pp. 33–7.

King, W. V. 1954. Chemicals evaluated as insecticides and repellents at Orlando, Fla. U. S. Dept. Agric. Handbk. **69**, 397 pp.

Lennette, Edwin H. 1955. Etiologic aspects of the infectious encephalitides. Proc. California Mosquito Control Assoc. and Amer. Mosquito Control Assoc. Conf., pp. 43–7.

Lindquist, A. W. 1955. Expanded grassland agriculture and the mosquito problem. Proc. California Mosquito Control Assoc. and Amer. Mosquito Control Assoc. Conf., pp. 23–5.

Lundstrom, Leon. 1954. Biological studies on *Culex tarsalis* (*Diptera: Culicidae*) in Kansas. Trans. Kansas Acad. Sci. **57**(1): 86–96.

Rapp, William F. 1955. *Culex tarsalis* and the grassland biome. Proc. California Mosquito Control Assoc. and Amer. Mosquito Control Assoc. Conf., pp. 89–90.

Smith, G. 1949. Kern MAD has circumstantial evidence on DDT resistance. (Monthly News-Sheet, California Mosquito Control Assoc.) Mosquito Buzz **3**(8): 2.

Stage, H. H., C. M. Gjullin, and W. W. Yates. 1952. Mosquitoes of the Northwestern States. U. S. Dept. Agric. Handbk. **46**. 95 pp.

Stivers, John O. 1956. Phosphate use in California mosquito control. Mosquito News **16**(2): 87–91.

BOOK REVIEWS

ADVANCES IN PEST CONTROL RESEARCH, VOLUME 1, Edited by R. L. Metcalf, Interscience Publishers Inc., New York and London, 1957, 6.25×9 inches, 514 pages, $11.00.

This book is a compilation of comprehensive reviews by specialists in various fields of pest control research. One chapter each is devoted to the following subjects: Control of Health Hazards Associated With the Use of Pesticides, Chemistry and Mode of Action of Herbicides, Use of Radioisotopes in Pesticide Research, The Chemistry and Action of Organic Phosphorus Insecticides, Mechanisms of Fungitoxicity, Recent Advances in Control of Soil Fungi, Repellents for Biting Arthropods, The Status of Systemic Insecticides in Pest Control Practices, Chemical Analysis of Pesticide Residues, and Bioassay of Pesticide Residues.

This volume contains a great deal of very useful information and is very well written. However, there is some inconsistency in the chemical nomenclature of several insecticides, a matter that might be confusing to some workers. Researchers in most aspects of economic biology will find this work a useful reference, especially the concise consideration of appropriate subjects, and the broad coverage of the literature. The chapters on Radioisotopes, Organic Phosphorus Insecticides, and Analysis of Pesticide Residues are particularly noteworthy and the contributors are to be commended. The volume is well printed on good quality paper and is nearly free of typographical errors. I consider this work a valuable contribution on a subject which is becoming increasingly important.

J. C. KELLER

(*Continued on page 116*)

Metabolism of Some Organophosphorus Insecticides by Microorganisms[1]

MOSTAFA KAMAL AHMED[2] and J. E. CASIDA, *Department of Entomology, University of Wisconsin, Madison*

ABSTRACT

The metabolism by some soil microorganisms of Thimet, Am. Cyanamid 12008, their oxidation products and certain other organophosphates was investigated. The yeast, *Torulopsis utilis*, the bacteria, *Pseudomonas fluorescens* and *Thiobacillus thiooxidans*, and the green alga, *Chlorella pyrenoidosa* were grown in pure cultures under aseptic conditions. The first two organisms were grown in a glucose-yeast extract medium and the latter two in mineral solutions. Rate studies were made on the hydrolysis and oxidation of organophosphates introduced into the cultures as emulsions at 1,000 p.p.m.

Torulopsis and *Chlorella* reacted similarly with the thiophosphates. It appeared as if the organophosphates were rapidly absorbed by the organisms, and then slowly released from the living and dead cells in the cultures. With both organisms the S-(alkylthio)methyl derivatives were much more rapidly hydrolyzed than the S-(alkylsulfonyl)methyl, and the latter only slightly faster than the S-(alkylsulfinyl)methyl compounds. In all cases the phosphorodithioate sulfoxides were the most stable and the phosphorothiolate sulfides were the least stable derivatives in the presence of the organisms. With *Chlorella*, Thimet was oxidized to the phosphorodithioate sulfoxide, which was very stable to hydrolysis but slowly converted to the phosphorothiolate sulfoxide with little if any phosphorothiolate sulfide or sulfones being formed. Both *Torulopsis* and *Chlorella* oxidized the sulfides to sulfoxides, but *Chlorella* was more effective in oxidizing the phosphorodithioates to phosphorothiolates. Little if any oxidation was found with parathion, Dow ET-57, dimefox and schradan in *Chlorella* cultures.

The two bacteria failed to oxidize Thimet but were effective at hydrolyzing this compound. *Thiobacillus* could not utilize sulfur from the Thimet molecule.

Metabolism of organophosphorus insecticides has been extensively studied in insects, mammals and plants. In their recommended usages, these insecticides frequently come in contact with the soil. Little information is available on their breakdown by microorganisms, particularly those of importance in the soil.

Mounter *et al.* (1955) demonstrated the presence in lyophilized bacterial cells of a phosphatase which hydrolyzed dialkylfluorophosphates with the liberation of fluoride ions. He further showed the properties of these phosphatases to vary with different bacteria, since they differed in their response to metal ion activators and inhibitors. Wäckers (1955) showed that the green algae, *Ankistrodesmus braunii* (Näg.) Collins, could store demeton inside their cells and liberate this organophosphate into demeton-free nutrient solutions. Cells rich in fat and deficient in nitrogen stored about 30% more demeton than the normal cells. Wäckers further demonstrated that the phosphorothioate demeton isomer doubled the respiration rate of both the algae *Ankistrodesmus* and *Chlorella* at a concentration at which the phosphorothiolate demeton isomer did not influence the oxygen consumption.

This study concerns the metabolism of certain organophosphorus insecticides by several soil microorganisms.

METHODS AND MATERIALS.—The organisms used in this study were two bacteria, *Pseudomonas fluorescens* Migula and *Thiobacillus thiooxidans* Waksman and Joffe, the yeast, *Torulopsis utilis* (Henneberg) Lodder, and the green alga *Chlorella pyrenoidosa* (Emerson's strain). All organisms were used in pure cultures only.

P. fluorescens is an aerobic bacterium which is quite abundant in soils and plays an important role in the decomposition of carbohydrates and proteins (Waksman, 1932). *Thiobacillus* is present in all normal soils, but is most active where sulfur-containing fertilizers are used (Waksman 1932). *Torulopsis utilis* has been isolated from many different types of soils and is present in Wisconsin soils (based on records of the Bacteriology Department of the University of Wisconsin). *T. utilis* is used as a supplement in cattle and human diets because of its ability to grow rapidly on the hydrolysate of waste wood (Underkofler & Hickey 1954) and the high protein content of the dried yeast, averaging 45 to 50% (Thaysen 1943). The *Chlorella* group has been frequently isolated from soils. Bristol Roach (1927) recognized the presence of two forms of *Chlorella* in English soils and found that this genus made up 35% of the common 10 species of algae in the upper 6 to 12 inches of soil. Moore & Carter (1926) observed *Chlorella* as far down as 5 feet in Missouri Botanical Garden soils. It has also been reported from south German and Danish soils (Waksman 1932).

For bacteria and yeast cultures, 50-ml. quantities of medium were placed in 125-ml. Erlenmeyer flasks which were plugged with cotton and sterilized by autoclaving for 1 hour at 15 lbs. After cooling, each flask was inoculated under aseptic conditions with 1 ml. of the pure culture suspension. The flasks were then shaken for 18 hours with the *Torulopsis* and *Pseudomonas* and for 2 to 3 days with the *Thiobacillus* to provide aeration as the original inoculum grew into a heavy population before introducing the organophosphates. For the larger culture volumes needed to yield sufficient metabolites for isolation and characterization, 200 ml. of medium were used in 500-ml. Erlenmeyers.

Torulopsis was grown in a medium of 2.0% glucose and 0.5% yeast extract in distilled water. This medium kept the cultures at about pH 6.0. At the time the organophosphates were added there was about 5.2 mg. of dry *Torulopsis* cells/ml. The growth progressed until the fourth day when 7.2 mg./ml. were present and then declined so that after 8 days only 4.7 mg. dry cells/ml. remained. In such determinations the cells were separated from the medium and washed by centrifugation. *Thiobacillus* was grown in the inorganic medium described by

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported in part by a research grant from the American Cyanamid Company. Accepted for publication July 29, 1957.

[2] Present address: Department of Plant Protection, University of Cairo, Giza, Egypt. The authors wish to acknowledge the generous help of Drs. Elizabeth McCoy and G. C. Gerloff of the University of Wisconsin in organizing and carrying out these studies, and of Dr. R. L. Starkey of Rutgers University for furnishing the *Thiobacillus* cultures.

Vogler & Umbreit (1941) consisting of 300 p.p.m. NH_4Cl, 3,000 p.p.m. KH_2PO_4, 250 p.p.m. $CaCl_2$, 500 p.p.m. $MgSO_4$, and 10 p.p.m. $FeSO_4$. Colloidal sulfur was sterilized separately for 3 hours with flowing steam and 0.5 gm. was added to each flask. The *Thiobacillus* cultures were held at pH 5.0 by adding sterilized Na_2CO_3 solution because of reduced *Thiobacillus* activity at higher pH values. *Pseudomonas* was grown in a culture medium of 0.5% yeast extract, 0.5% glucose and 0.7% KH_2PO_4, a medium which kept the pH between 5.5 and 6.2 during the experiment. In the 8-day experimental period, the *Pseudomonas* population dropped from about 2.3 mg. dry cells/ml. to 0.82 mg./ml.

Chlorella was grown by a method (Gerloff 1956) for which the apparatus is illustrated in figure 1. The cultures were aerated with a mixture of air filtered through cotton (A) and CO_2, the composition of the mixture being adjusted to 5% CO_2 as measured by a manometer (B). The CO_2 was washed with water (C) before mixing with the air (D) and passing through another filter (E) packed half with cotton to absorb the excess moisture and half with glass wool to pick up oil droplets which might pass through the system from the air compressor. The 5% mixture of CO_2 in air then passed through "T" and capillary tubes (H and J) adjusted in capillary size and length to allow the same rate of flow for the gas mixture into each of the 1.5×12 in. culture tubes (F). Rubber tubing thoroughly washed with distilled water was used for all non-glass connections. The culture tubes (F) were mounted in a stainless steel rack (G) and illumination provided by two 40-watt tubular fluorescent bulbs on either side of the rack. *Chlorella* was grown in Knop's solution as modified by Myer & Clark (1944) consisting 3792 p.p.m. KNO_3, 2456 p.p.m. $MgSO_4 \cdot 7H_2O$, 1,25 p.p.m. KH_2PO_4, 74 p.p.m. $CaCl_2 \cdot 2H_2O$, 30 p.p.m. ferric citrate and 30 p.p.m. of citric acid. Pyrex distilled water was used throughout and Hoagland's A_5 solution (Hoagland & Arnon 1950) was added to provide 9.5 p.p.m. B, 9.5 p.p.m. Mn, 0.02 p.p.m. Zn, 0.02 p.p.m. Cu and 0.01 p.p.m. Mo. The culture medium was adjusted to pH 6.8 and 150 ml. added to each tube (F), which was then plugged with cotton and the series of tubes connected as indicated in figure 1. A clamp closed the rubber tubing at position "I" to prevent the nutrient solutions from sucking from one tube to another from pressure changes on autoclaving. The system of tubes in the rack along with the cotton-glass wool filter (E) was autoclaved for 20 minutes at 15 lbs., cooled, the clamps at position "I" re-

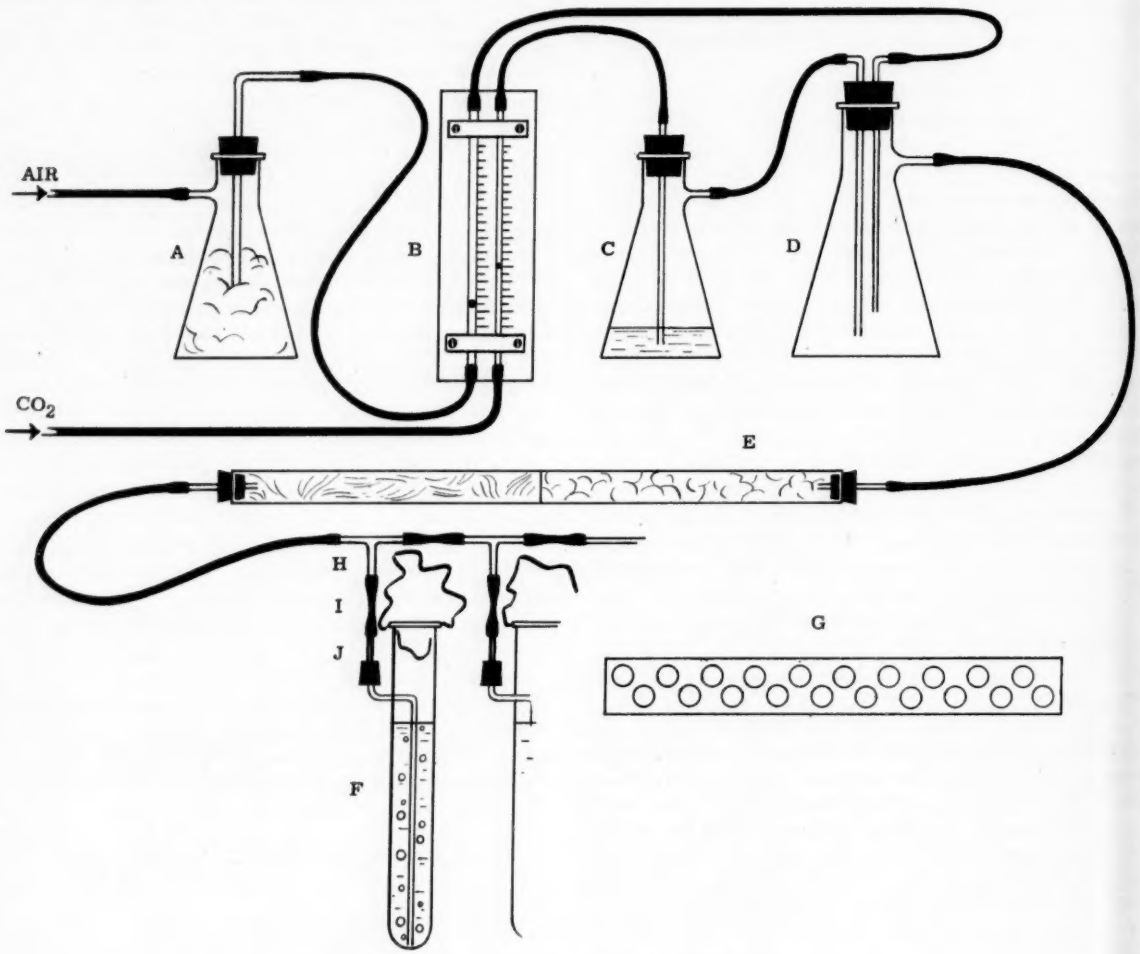


FIG. 1.—Aeration apparatus used for *Chlorella* cultures.

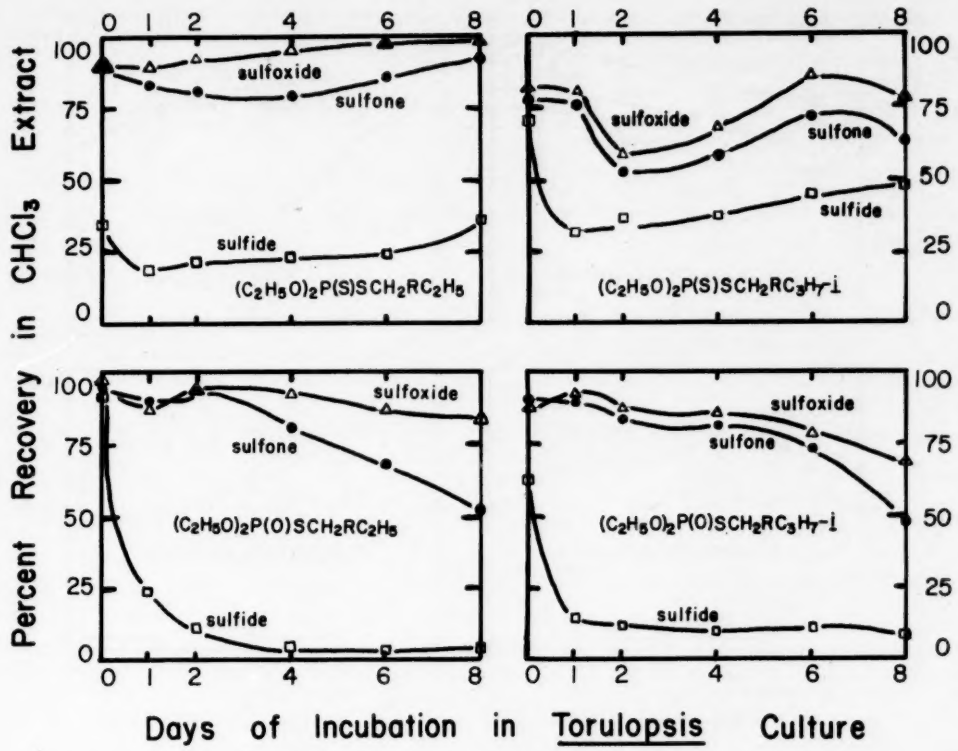


FIG. 2.—Metabolism of Thimet and Am. Cyanamid 12008 derivatives in *Torpulopsis* cultures.

moved and each tube inoculated under aseptic conditions with a 1.0 ml. suspension of *Chlorella* cells. The remaining parts of the aeration equipment were then connected and the gas mixture passed through the cultures at the rate of 1.5 liters of gas/min./liter of medium. After 4 to 6 days growth when the cell population was about 1.2 to 1.5 mg. of dry cells per ml. of culture, a small drop of Antifoam KCM (Hodag Chemical Co., Chicago, Illinois) was introduced into each tube by a sterilized bacteriological transfer needle to minimize foaming in the cultures. After 8 days of metabolism time for the organophosphates, when the experiment was terminated, there were about 4.0 mgm. of dry *Chlorella* cells per ml. of medium. Similar determinations in the presence of the compounds showed that none of the materials at the 1,000 p.p.m. level used affected the growth rate of the algal cultures.

Emulsifiable concentrates of the organophosphates were prepared by mixing equal weights of the phosphates with Triton X-155 and emulsifying in sterilized distilled water. The emulsifiable concentrates were introduced under aseptic conditions into the cultures to give a final concentration of 1,000 p.p.m. for each the emulsifier and the compound. Samples of the cultures from 3.0 to 5.0 ml. each were harvested with sterile volumetric pipettes at 0, 1, 2, 4, 6 and 8 days after introducing the compounds and the samples were extracted with equal volumes of chloroform in 15 ml. tubes with centrifugation to aid the separation of the solvent phases. Aliquots of the chloroform extracts were then analyzed for total phosphorus by the method of Allen (1940). Direct analyses were made on the emulsifiable concentrates for total phosphorus to calculate the original amount of organophosphate introduced into the culture, so that the per cent of compound recovered and that lost due to hydrolysis or binding in the cells could be calculated. At the end of the 8-day experimental periods, the remainder of the cultures were extracted with chloroform and the partitioning of the chloroform-soluble phosphorus compounds determined between hexane, acetone and water in a volume ratio of 5:4:7. The chloroform-soluble materials from larger cultures were chromatographed on celite columns using the technique described by Bowman & Casida (1957). Fractions from these columns were located by total phosphorus and anticholinesterase analyses and characterized by their infra-red spectra (10% chloroform solutions of the organophosphates in a sodium chloride prism with a Baird spectrophotometer). The techniques used in studying the phosphoramides were those described by Arthur & Casida (1957).

The compounds used were several dialkyl phenylphosphates and phosphorothioates, dimefox, schradan, Thimet, Am. Cyanamid 12008 (common names based on Haller 1957) and the sulfinyl and sulfonyl derivatives of Thimet and Am. Cyanamid 12008 and their phosphorothiolate analogs.

RESULTS AND DISCUSSION.—The yeast, *Torulopsis utilis*, and the alga, *Chlorella pyrenoidosa*, were quite similar in their action on Thimet, Am. Cyanamid 12008, their phosphorothiolate, sulfinyl and sulfonyl derivatives. Considerable amounts of the compounds were unextractable soon after introducing them into the cultures (figures 2 and 3). This inability to recover the added organophos-

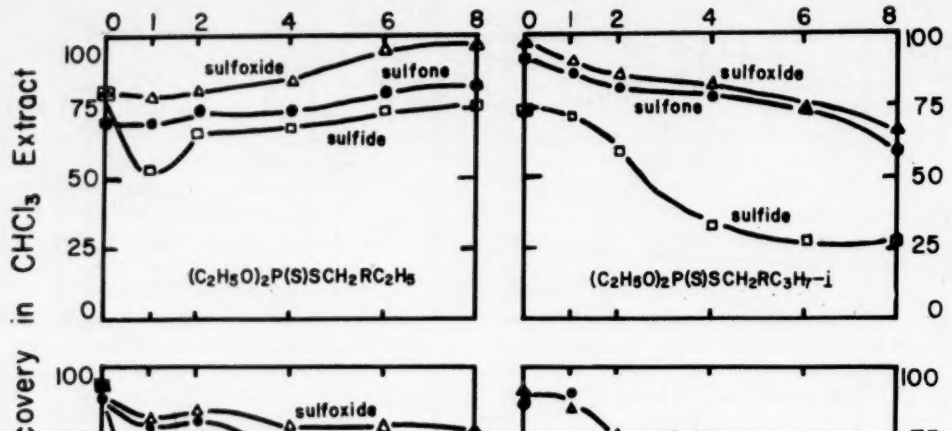


FIG. 3.—Metabolism of Thimet and Am. Cyanamid 12008 derivatives in *Chlorella* cultures.

phates was more evident with the sulfides than with the sulfoxides and sulfones, and was most pronounced with Thimet added to *Torulopsis* cultures where only 34% of the Thimet could be recovered by chloroform extraction immediately after addition. In certain cases the absorbed materials were later released from the living and dead cells in the culture. Wäkers (1955) found that demeton was stored by *A. braunii* and then liberated into demeton-free nutrient solutions.

Both *Torulopsis* and *Chlorella* hydrolyzed many of the Thimet and Am. Cyanamid 12008 derivatives, with the *Chlorella* being somewhat more effective (figures 2 and 3). The sulfides were attacked much more readily than the sulfones, and the latter only slightly faster than the sulfoxides. The phosphorothiolates were always less stable than the phosphorodithioates, the greatest difference occurring with their respective sulfides. In all cases the phosphorodithioate sulfoxides were the most stable and the phosphorothiolate sulfides the least stable derivatives in the presence of the organisms.

Certain Thimet and Am. Cyanamid 12008 derivatives were subject to oxidation as well as hydrolysis. The solubility properties of the residual organophosphates after metabolism by the organisms indicated the formation of more polar oxidation products (table 1). *O,O*-diethyl *S*-(alkylthio)methyl phosphorodithioates and phosphorothiolates were recovered from *Chlorella* and *Torulopsis* cultures mixed with their sulfoxides. Twenty-two per cent of the Thimet incubated 8 days in the yeast cultures was recovered as *O,O*-diethyl *S*-(ethylsulfinyl)-methyl phosphorodithioate based on column chromatography of the metabolites and infra-red and partition coefficient determinations on the fractions obtained. No phosphorothiolate derivatives and sulfones were detected from the Thimet metabolized by the yeast. *O,O*-diethyl *S*-(ethylsulfinyl)methyl phosphorothiolate incubated with *Torulopsis* was recovered as the same compound in the chloroform-soluble fraction (within the sensitivity of the column chromatography and infra-red absorption techniques). Seventy-eight per cent of Thimet and its chloroform-soluble metabolites were recovered from a *Chlorella* culture, and column chromatography, partition coefficients and infra-red spectra on the metabolites indicated that 12.3% *O,O*-diethyl *S*-(ethylsulfinyl)methyl phosphorodithioate and 2.9% *O,O*-diethyl *S*-(ethylsulfinyl)-methyl phosphorothiolate were formed with less than 1% total of the other three possible oxidation products being formed. *O,O*-Diethyl *S*-(ethylsulfinyl)methyl phosphorodithioate was recovered without *in vivo* oxidation after 8 days in *Chlorella* culture based on the sensitivity of the column chromatography and infra-red techniques. Similarly incubation of the phosphorothiolate sulfoxide with *Chlorella* formed little if any phosphorothiolate sulfone based on infra-red spectra. These studies considered in relation to the information in table 1 indicate that with *Chlorella* the phosphorodithioate sulfide (Thimet) was oxidized to the phosphorodithioate sulfoxide, which was very stable to hydrolysis but slowly converted to the phosphorothiolate sulfoxide with little if any phosphorothiolate sulfide or sulfones being recovered. The phosphorothiolate sulfide was rapidly hydrolyzed and slowly oxidized to the phosphorothiolate sulfoxide. Torulopsis reacted nearly the same as *Chlorella* (table 1) but was less effective in oxidizing the phosphorodithioate to the phos-

Table 1.—Partitioning properties of metabolites from Thimet, Am. Cyanamid 12008 and their oxidation products after 8 days' incubation in cultures of *Torulopsis* and *Chlorella*.[a]

Compound	Thimet Derivatives (R = ethyl)			Am. Cyanamid 12008 Derivatives (R = isofropyl)		
		Metabolites from			Metabolites from	
	Known	*Torulopsis*	*Chlorella*	Known	*Torulopsis*	*Chorella*
$(C_2H_5O)_2P(S)SCH_2SR$	100	55	48	100	83	89
$(C_2H_5O)_2P(S)SCH_2S(O)R$	30	34	48	55	55	37
$(C_2H_5O)_2P(S)SCH_2S(O)_2R$	57	60	55	68	68	58
$(C_2H_5O)_2P(O)SCH_2SR$	62	21	38	83	60	14
$(C_2H_5O)_2P(O)SCH_2S(O)R$	2	—	—	9	—	—
$(C_2H_5O)_2P(O)SCH_2S(O)_2R$	4	—	—	8	—	—

[a] All figures represent the per cent of total phosphorus partitioning in the hexane layer with a hexane, acetone, water mixture in a volume ratio of 7:5:4.

phorothiolate. Thimet and Am. Cyanamid 12008 derivatives responded similarly in respect to oxidative metabolism by *Chlorella* and *Torulopsis*.

It is interesting to note that the metabolism of Thimet and Am. Cyanamid 12008 by the *Chlorella* and *Torulopsis* was similar except for the extent of oxidation to that reported for Thimet, Bayer 19639, and Systox in higher plants (Fukuto *et al.* 1956, Bowman & Casida 1957, Metcalf *et al.* 1957).

Several dialkyl phenylphosphates were incubated with *Torulopsis* cultures for 8 days. From 45 to 65% of the following materials were recovered after this metabolism time: methyl parathion, Am. Cyanamid 4124 and its oxygen analog, Chlorthion, and Dow ET-57 and its oxygen analog.

After 8 days incubation in a *Chlorella* culture, parathion was recovered 37% as chloroform-soluble materials. Chromatography and infra-red determination of the recovered organophosphate failed to indicate the formation of para-oxon. Dow ET-57 was 72% recovered from *Chlorella* cultures after 8 days, but under the test conditions the Dow ET-57 was in excess of the saturation point for the culture and crystallization occurred within 24 hours. Of the chloroform-solubles recovered greater than 98% of the organophosphate was ET-57 *per se* based on chromatography and infra-red spectra. Under similar conditions with *Chlorella*, schradan was recovered to the extent of 71% and dimefox 84% after 8 days. The *Chlorella* formed no chloroform-soluble metabolites that yielded formaldehyde on acid degradation from either dimefox or schradan nor formed any metabolites differing from the original phosphoramides in solvent partitioning, chromatographic characteristics or infra-red spectra (techniques after Arthur & Casida 1957).

Pseudomonas cells hydrolyzed 58% of the Thimet incubated with them for 8 days but no oxidation occurred at any site on the organophosphate molecule since the residual organophosphate recovered partitioned completely into hexane from an acetone-water mixture.

Thiobacillus hydrolyzed 75% of the Thimet within 8 days and the residual organophasphate recovered was Thimet *per se*. Although *Thiobacillus* requires sulfur for growth, it failed to utilize the sulfur from the Thimet molecule. When Thimet was added to the *Thiobacillus* medium as the only energy source, the introduced inoculum failed to grow, but when elemental sulfur was added to these cultures, a heavy *Thiobacillus* population was rapidly formed.

References Cited

Allen, R. J. L. 1940. The estimation of phosphorus. Biochem. Jour. **34:** 858–65.

Arthur, B. W., and J. E. Casida. 1957. Biological and chemical oxidation of tetramethyl phosphorodiamidic fluoride (dimefox). Jour. Econ. Ent. (In press)

Bowman, J. S., and J. E. Casida. 1957. Metabolism of the systemic insecticide *O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate (Thimet) in plants. Jour. Agric. Food Chem. **5:** 192–7.

Bristol Roach, B. M. 1927. On the algae of some normal English soils. Jour. Agric. Sci. **17:** 563–88.

Fukuto, T. R., J. P. Wolf, III, R. L. Metcalf and R. B. March. 1956. Identification of the sulfoxide and sulfone plant metabolites of the thiol isomer of Systox. Jour. Econ. Ent. **49:** 147–51.

Gerloff, G. C. 1956. Personal communication.

Haller, H. L. 1957. Common names of insecticides. Jour. Econ. Ent. **50:** 226–8.

Hoagland, D. R., and D. I. Arnon. 1950. The water-culture method for growing plants without soil. California Expt. Sta. Circ. **347:** 31 pp.

Metcalf, R. L., T. R. Fukuto and R. B. March. 1957. Plant metabolism of dithio-Systox and Thimet. Jour. Econ. Ent. **50:** 338–45.

Moore, G. T., and N. Carter. 1926. Further studies on the subterranean algal flora of the Missouri Botanical Gardens. Ann. Missouri Bot. Garden **13:** 101–40.

Mounter, L. A., R. F. Baxter and A. Chanutin. 1955. Dialkylfluorophosphatases of microorganisms. Jour. Biol. Chem. **215:** 699–704.

Myer, J., and L. B. Clark. 1944. Culture conditions and the development of the photosynthetic mechanism. II. An apparatus for the continuous culture of *Chlorella*. Jour. Gen. Physiol. **28:** 103–12.

Thaysen, A. C. 1943. Value of micro-organisms in nutrition (food yeast). Nature **151:** 406–8.

Underkofler, L. A., and R. J. Hickey. 1954. Industrial fermentations. Vol. I. Chem. Publ. Co. Inc. N. Y.

Vogler, K. G., and W. W. Umbreit. 1941. The necessity for direct contact in sulfur oxidation by *Thiobacillus thiooxidans*. Soil Sci. **51:** 331–7.

Wäckers, R. W. 1955. Phytophysiological effects of the systemic insecticide Systox (diethyl thionophosphoric ester of B-oxyethyl thioethyl ether) Höfchen-Briefe (English edition) Bayer Leverkusen, Germany. **8:** 265–324.

Waksman, S. A. 1932. Principles of soil microbiology. The Williams and Wilkins Co., Baltimore, Md.

Effects of Flight Distances from Honey Bee Colonies on Red Clover Seed Yields[1]

R. J. WALSTROM[2,3]

ABSTRACT

Experiments on second crop red clover seed yields at various distances from honey bee colonies carried out in Greene County, Iowa, from 1951 to 1953, inclusive, gave the following results:

1. Differences in seed yields at 100-foot intervals from honey bee colony sites during the 3-year period were significant at the 1% level of probability.
2. In 1951 and 1952 the difference in seed yield within the 100- to 400-foot range from the colonies compared with the yield in the remainder of the field was significant at the 1% level of probability. Larger seed yields occurred within the 100- to 400-foot range.

The effects of flight distance on the pollen-gathering activities of honey bees (*Apis mellifera* L.) apparently have not been critically studied until recently. Alpatrov (1940) reviewed a Russian report which indicated that the distance travelled for red clover pollination varied among the three races of honey bees used in the test. Butler (1943) reported that on any given crop the greatest numbers of foraging bees were found nearest to the hives.

Ribbands (1949) noted that individual foraging bees would confine themselves to particular areas. He reported that bees would change from pollen gathering to nectar gathering, but the reverse shift was never observed. Ribbands (1951) commented that honey bee colonies should be placed as close to the crop as possible to obtain maximum pollinating efficiency. He stated that the amount of foraging during unfavorable weather was considerably reduced when the bees had to fly even short distances.

MacVicar *et al.* (1952) reported that the maximum yield of red clover seed was produced 500 yards away from 68 colonies placed on the south edge of the field. However they also noted a comparatively poor stand at the 200-yard distance, the only sampled area between colonies and the 500-yard distance.

Braun *et al.* (1953) carried out tests on red clover fields using two colonies per acre. They found that the number of honey bee pollinators per unit area decreased as the distance from the apiary increased. They reported the highest seed yields where the pollinator numbers had been the greatest, and stated that seed yield decreased as the distance from the apiary increased. Pollen analyses of collections from traps on colonies in the field indicated that red clover pollen made up from 11 to 71% of the total.

Table 2.—Analyses of variance of red clover seed yields obtained at different distances from colony sites in Greene County, Iowa, from 1951 to 1953, inclusive.

Source of Variation	Degrees of Freedom	Mean Squares	F.
	1951		
Distance			
100–400 feet vs. balance	1	1,533.40	456.37**
within 100–400 feet	3	29.96	8.92**
beyond 400 feet	7	15.12	4.50
	11	156.65	46.62**
Samples	108	3.36	
	1952		
Distance			
100–400 feet vs. balance	1	1,700.30	117.34*
within 100–400 feet	3	68.10	4.70**
beyond 400 feet	8	29.10	2.01*
	12	178.12	12.29**
Samples	117	14.49	
	1953		
Distance			
100–400 feet vs. balance	1	20.25	2.23
within 100–400 feet	3	2.62	1
beyond 400 feet	4	56.51	6.23**
	8	31.82	3.50**
Samples	81	9.08	
	1951 through 1953 inclusive		
Years	2	1,419.51	2.13
Distance	8	1,957.87	2.93*
Interactions	16	667.27	

* Significant at 5% level of probability.
** Significant at 1% level of probability.

METHODS.—This study was conducted to determine the effects of honey bee pollinating activity on red clover seed production under central Iowa conditions. The results were expected to provide information as to the most

[1] Journal Paper No. J-3235 of the Iowa Agricultural Experiment Station, Ames. Project No. 1001. Accepted for publication August 1, 1957.

[2] Now Associate Entomologist at South Dakota State College, Brookings.

[3] The advice and assistance of Professors F. B. Paddock, and J. H. Lilly are gratefully acknowledged.

Table 1.—Populations of major injurious insects prior to the application of insecticides on red clover fields at Paton in Greene County, Iowa. 1951 to 1953, inclusive.

Year	Injurious Insects (Number per Sweep)						
	Plant Bugs			Grasshoppers	Clover Head Caterpillar	Pea Aphid	Lesser Clover Leaf Weevil
	Lygus	Rapid	Alfalfa				
1951	2.6	0.7	0.3	2.4	0.1	0.7	0.1
1952	1.9	0	0.1	1.2	0	2.7	0.4
1953	1.2	0.7	1.1	1.7	0.2	1.1	0.9

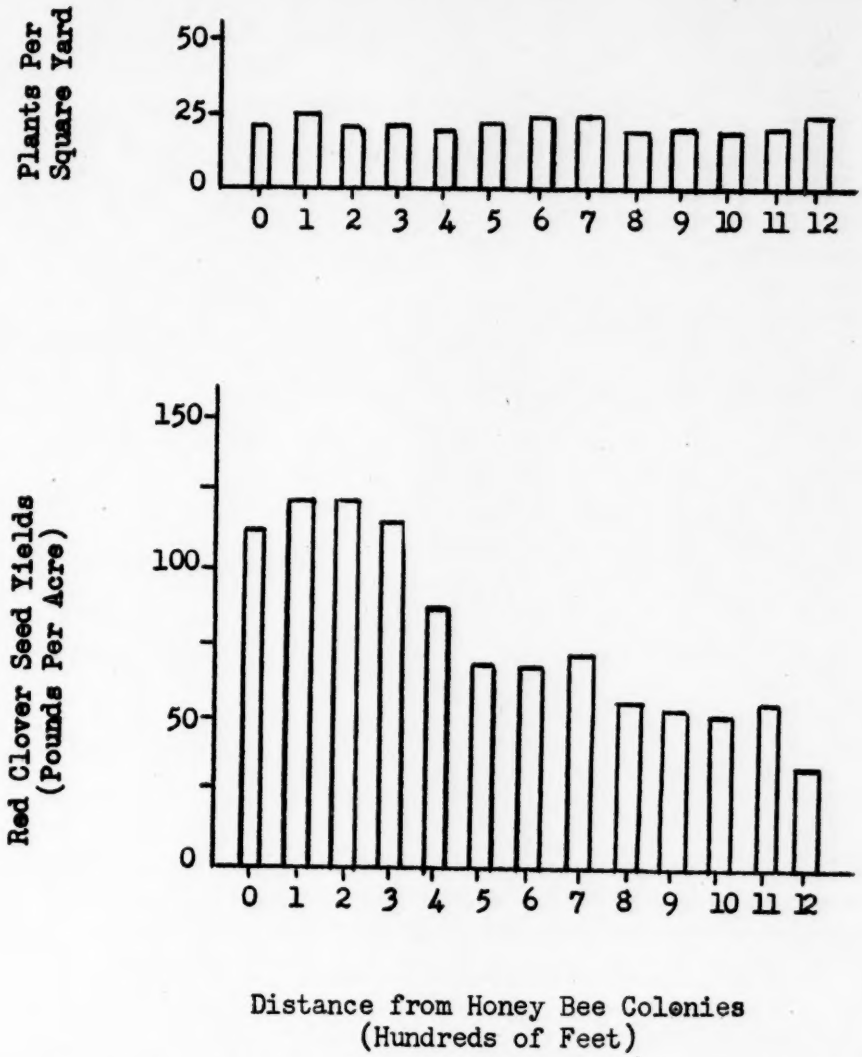

Plants Per Square Yard
50
25
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Red Clover Seed Yields (Pounds Per Acre)
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Distance from Honey Bee Colonies (Hundreds of Feet)

FIG. 1.—Red clover plant population and seed yield variations as related to distance from honey bee colonies. Greene County, Iowa. 1951.

desirable locations for pollinating colonies in red clover fields.

Studies of the relationship of honey bee flight distances to red clover seed yields were conducted on the T. E. Smith farm 1 mile north of Paton in Greene County, from 1951 to 1954 inclusive. Colonies with strengths of eight or more frames of brood were provided at blooming time at the rate of two per acre. In 1951 the colonies were placed on the north edge of the seed-producing field. They were rotated to the east edge in 1952 and to the south edge in 1953. A west edge location was planned for 1954 but a poor stand of red clover and alsike competition made this location unsatisfactory.

Pest insects were controlled by applying a spray of 1½ pounds of actual toxaphene per acre when the plants were in the bud stage. Table 1 shows the major injurious insect populations at the time of spraying for each of the 3 years.

Ten yard-square samples were cut for seed directly in front of and at 100-foot intervals across the field from the honey bee colonies. These samples were placed in paper bags and taken to Ames where they were dried in one of the College greenhouses. At the same time the samples were harvested, counts were made to determine the number of plants per square yard (figures 1, 2, and 3).

Soil samples were taken at 200-foot intervals throughout the length of the field to discover any soil variations which might help explain the differences in seed yields. These samples were tested for soil pH, available phosphorous, and available potassium by the Soil Testing Laboratory of Iowa State College.

The seed was threshed from the bagged samples by first running the samples through a McCormick Deering 4.E hammermill with a no. 8 screen. The samples were then run thrugh an A. T. Ferrell Office Clipper fanning mill with a 1/12-inch screen. Where foreign matter such as timothy seed remained as contamination the samples were further cleaned by hand. This hand cleaning process

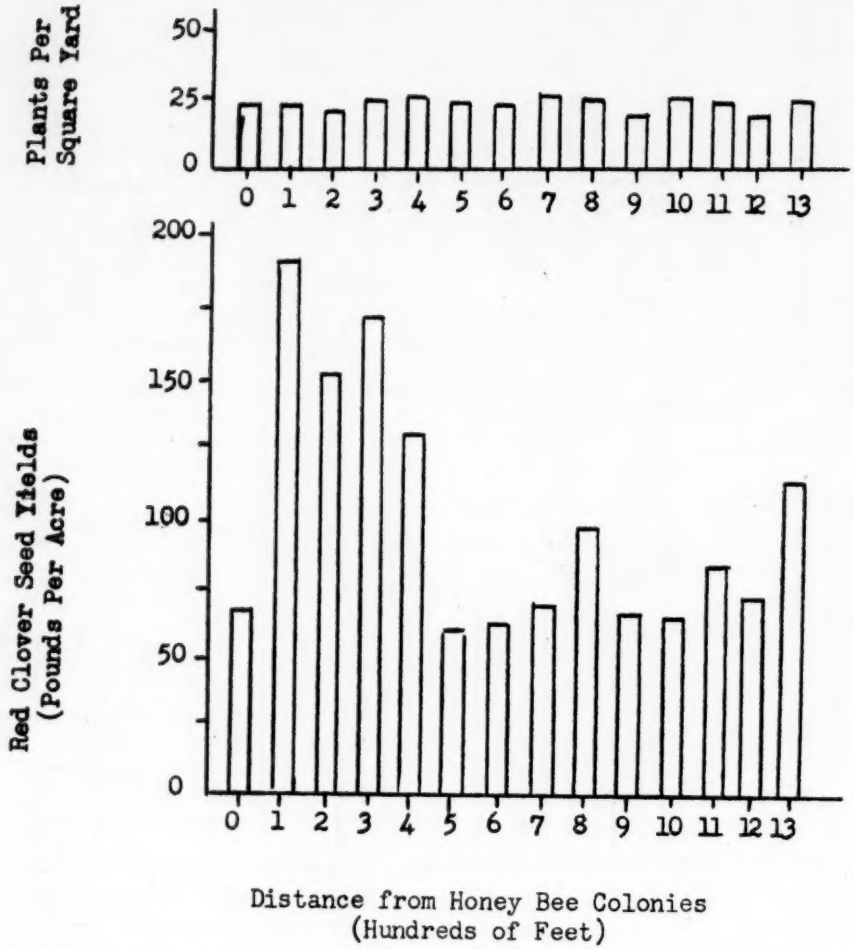

Plants Per Square Yard
50
25
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Red Clover Seed Yields (Pounds Per Acre)
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Distance from Honey Bee Colonies (Hundreds of Feet)

FIG. 2.—Red clover plant population and seed yield variations as related to distance from honey bee colonies. Greene County, Iowa. 1952.

was accomplished most satisfactorily by spreading a sample on a flat surface and pressing it with a wooden block covered with a layer of thick velvet cloth. The samples were then weighed to the closest hundreth of a gram. Where comparisons with field yields were desired, the grams per square yard were converted to pounds per acre by multiplying by the appropriate conversion factor, 10.67. Seed yields for the 3 years at the various distances from the honey bee colonies are shown in figures 1, 2, and 3.

Information on weather conditions through the season was obtained from the nearest United States Weather Bureau Station located 1 mile south of Jefferson, Iowa. These records included precipitation, and daily maximum and minimum temperature readings.

RESULTS.—Red clover seed yields varied at different distances from honey bee colonies in the various experimental fields from 1951 through 1953 inclusive.

In 1951 when the colonies were placed on the north edge of a field, the differences in seed yields at different distances from the colonies were significant at the 1% level of probability (table 2). The difference in seed yield within the 100- to 400-foot range compared with the yield in the remainder of the field was also significant at the 1% level of probability.

Plant conditions at the time of harvesting were generally good, except that the areas near the colonies from which the first set of samples were taken invariably showed evidence of trampling from colony manipulations. In the 1953 field the area from the colonies to a distance of 170 feet was completely lodged when harvested.

The plant populations (figures 1, 2, 3) and the soil fertility levels within the fields showed no differences which can be presumed to have caused the variations of seed yields. In general the soil pH and the available potassium levels for the fields were good, but a general deficiency in available phosphorous was found.

When the colonies were placed on the east edge of the field in 1952 the results were similar to those obtained in 1951. Differences in seed yields correlated with distances from the colonies were significant at the 1% level of probability (table 2). The seed yields within the 100- to 400-foot range compared with the yields for the remainder of the field were also significantly different at the 1% level of probability.

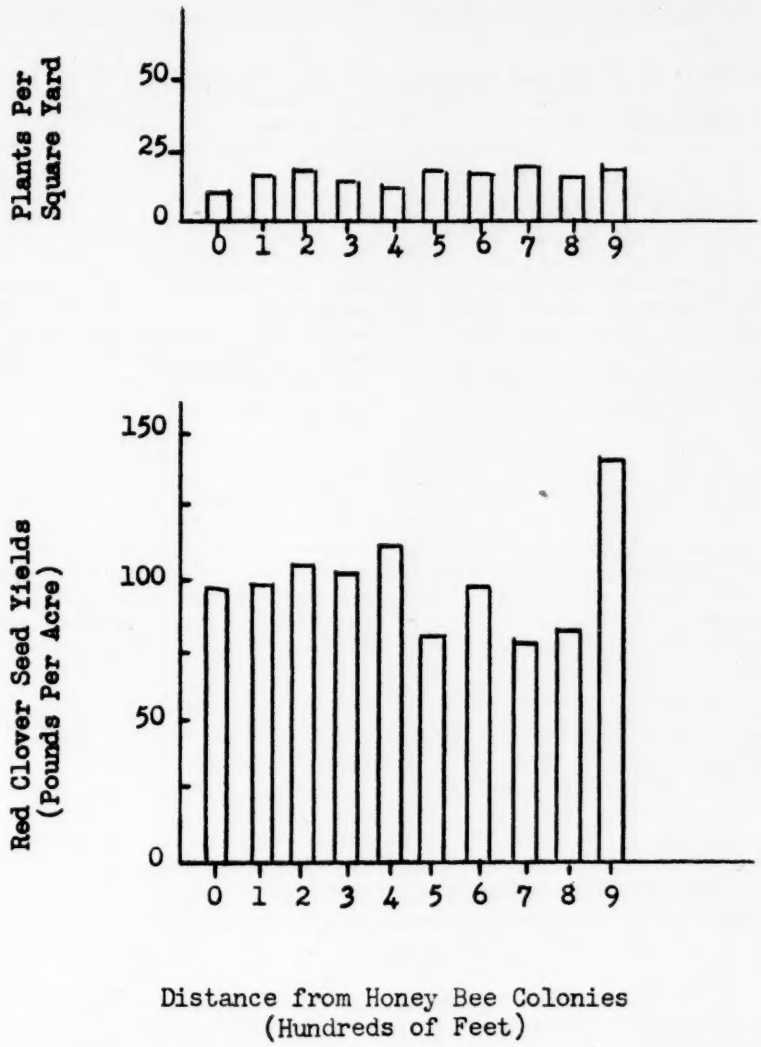


FIG. 3.—Red clover plant population and seed yield variations as related to distance from honey bee colonies. Greene County, Iowa. 1953.

When the colonies were placed on the south edge of the field in 1953 it was again found that differences in seed yields correlated with distances from the colonies were significant at the 1% level of probability (table 2). Severe plant lodging occurred up to 170 feet from the colonies in this field. The lodging apparently was caused by prevailing winds whipping around an adjacent grove of trees. This lodging, plus an unexplained high seed yield at the 900-foot distance, resulted in a non-significant difference between the yields for the 100- to 400-foot range and the remainder of the field.

A combined analysis of variance for the results over the 3-year period showed differences in seed yields correlated with distances from the colonies which were significant at the 5% level of probability.

It is concluded that honey bee colonies intended for red clover pollination should be placed in the field under central Iowa conditions so as to make the best use of the maximum pollination efficiency occurring within 400 feet of the colony locations.

REFERENCES CITED

Alpatrov, V. V. 1948. Bee races and red clover pollination. Bee World **29**: 61–62.

Braun, E., R. M. MacVicar, D. R. Gibson, P. Pankiw, and J. Guppy. 1953. Studies in red clover seed production. Part II. Canadian Jour. Agric. Sci. **33**: 437–447.

Butler, C. G. 1943. Work on repellents; Management of colonies for pollination. Ann. Appl. Biol. **30**: 189–97.

MacVicar, R. M., E. Braun, D. R. Gibson, and C. A. Jamieson. 1952. Studies in red clover seed production. Sci. Agric. **32**: 67–80.

Ribbands, C. R. 1949. The foraging method of individual honey bees. Jour. Anim. Ecol. **18**: 47.

Ribbands, C. R. 1951. The flight range of the honeybee. Jour. Anim. Ecol. **20**: 220.

Chemical Control Studies of the Wheat Curl Mite[1]

EDMON J. KANTACK[2] and HERBERT KNUTSON,[3] *Kansas State College, Manhattan*

ABSTRACT

Little work has been reported on the control of eriophyid mites with synthetic organic acaricides, and virtually none on the wheat curl mite, *Aceria tulipae* (Keif.). Rolling of the leaves caused by this species and the tendency for some mites to occur deep in the leaf sheaths between two surfaces protects them from sprays and suggested the translocation of systemic insecticides as the most promising means of control.

Spraying with 30 different insecticides in greenhouse and field in the spring resulted in none that gave both high initial and long residual control. Eighty to 90% control was obtained at 2 to 3 days in the field with several insecticides but none exceeded 90% control. Best control at 5 weeks following spraying of young wheat in the fall was obtained with endrin in light mineral seal oil.

After determining the maximum concentration of each systemic toxicant that was not highly detrimental to germination, three concentrations of each toxicant were tested. Retardation and/or reduction in germination resulted from the use of all seed treatments, although not above the 5% confidence limit in most cases when the toxicant did not exceed 0.125 pound per bushel. Impregnation of the toxicant in 4 pounds of Carbowax-6000 per bushel of seed, gave better control than soil drenches. Am. Cyanamid 12009 (95% control) and 12008 (85% control) at an average from 0.125 to 0.5 pound of toxicant in 4 pounds of Carbowax per bushel of seed, gave the best overall results at the end of 1 week. When these toxicants were impregnated in attaclay at 1.0 pound toxicant per bushel, 89 and 74% control, respectively, was obtained at 1 week and 98 and 97%, respectively, at the end of 5 weeks.

In field studies on plantings of fall-treated seed, best results, using Am. Cyanamid 12008 (89% control) and 12009 (72% control), were recorded at 5 weeks when the toxicant was used without being impregnated in granules. Mites were present in all cases when counts were made 10 weeks following planting as well as during the following spring.

In no instance was control of wheat-streak mosaic obtained from seed treatments in 1954 when viruliferous mites were constantly introduced in large numbers onto the young wheat plants.

The eriophyid mite, *Aceria tulipae* (Keif.), (Eriophyidae) is of major economic importance to wheat growers because of its role as a vector of wheat-streak mosaic virus (Slykhuis 1953). In the great plains area of the United States and Canada this disease is responsible for severe reductions in yield during some years. The most severe infection by mosaic in western Kansas was reported by Fellows (1949). Melchers (1950) estimated that the 1949 reduction in Kansas was 15½ million bushels, a $30,000,000 loss. In 1954, the estimated loss was $14,000,000 (Sill *et al.* 1955).

In Kansas the mites are often sheltered from insecticidal exposure within the leaf sheath, including the pocket formed by the ligule, as well as within the curled leaf (Kantack & Knutson 1954).

There has been relatively little work reported on chemical control of *A. tulipae*. Staples & Allington (1956), in greenhouse tests, using a dipping technique, found demeton and parathion more effective than Metacide, malathion, ovex and Aramite. Systox at "1 pint per acre" and parathion at "1 quart per acre" was sprayed on September 12, in a wheat streak mosaic area, at dilutions to make 15 gallons of spray material. At the time of spraying, the wheat was in the 2-leaf stage, having emerged 7 days previously. No disease control was obtained under these conditions.

MATERIALS AND METHODS.—Preliminary screening of insecticides[4] was conducted in the greenhouse, using Kiowa wheat. Infestation with mites was accomplished by inserting ¼-inch lengths of heavily infested leaves in the leaf axil of the basal leaf. Heavy infestations were obtained in 5 to 6 weeks. Greenhouse temperatures were maintained near 70° F. A fine mist of spray was applied to the entire exposed surface of the plant to a point of near run-off. Because of the paucity of data on chemicals effective against eriophyids, chemicals tested were not limited to the usual acaricides. Mite counts were made at 1-, 10-, 28-, and 40-day intervals on a measured area of the 1 leaf per plant which microscopically appeared to be most heavily infested.

Selections of chemicals for spring field spray tests were made from those showing greatest promise in the greenhouse. These were conducted on fall-sown Pawnee wheat during the spring jointing stage in a field which was virtually 100% infested with mites. Although sprayings were completed before head emergence began, mite survival counts were taken until the grain ripened. Sprays were applied on randomized, 1-square-rod plots, replicated four times, with similar control plots left unsprayed. A knapsack sprayer with a disc nozzle was used, which delivered at the rate of 20 gallons per acre. Counts were made at 2-, 10-, and 28-day intervals on April 27 on the first test plot. Mite counts on the second day were made on a measured leaf area of 24 plants on the leaf immediately below the boot, and on this area as well as the flag leaf during the 10- and 28-day counts. Counts were made at 3-, 10-, and 28-day intervals on May 10 on the second plot. Counts at 3 and 10 days were on a measured leaf area of 24 plants on the flag leaf. The 28-day counts were made on the whole crease side surface area of 24 kernels, on each of six heads taken randomly from each replicate. Mites at this time had just moved into the head

[1] Contribution No. 694, Department of Entomology, Kansas Agricultura Experiment Station, Manhattan. Accepted for publication Aug. 12, 1957.

[2] Formerly Graduate Research Assistant, Kansas State College, and now Assistant Professor, Entomology Research Department, Louisiana State University. Portion of a dissertation presented as partial fulfillment for the degree, Doctor of Philosophy in Entomology at Kansas State College.

[3] Professor of Entomology, Kansas State College, and Entomologist, Kansas Agricultural Experiment Station.

[4] The following insecticide emulsion concentrates were included in preliminary greenhouse screening but not tested further: aldrin, 1 lb./acre; Aramite, 0.5 lb./acre; DDT, 4 lbs./acre; EPN, 0.5 lb./acre; Nitrox (0,0-dimethyl 0-p-nitrophenyl phosphorothioate), 23% emulsion, 0.4 lb./acre, E. I. duPont de Nemours & Co.; Orthorix, (active ingredients 26% calcium polysulfides), 36% at 0.75 lb./acre, California Spray-Chemical Corp.; ovex, 50% wettable powder, 0.5 lb./acre.

region and showed heaviest populations within the glumes and particularly on the kernels.

The chemicals showing the most promise in spring tests, and some not available during the spring, were sprayed at 80 p.s.i. with a fan-type spray on fall-seeded Pawnee wheat 3 weeks after the planting date (2- and 3-leaf stage). Treatments were on plots 8 by 10 feet, arranged in randomized blocks replicated four times. Approximately 200 greenhouse-grown wheat plants, heavily infested with mites, were transplanted to the border rows 11 days after the plots were seeded. Two rows of plants located between the two center randomized blocks were likewise infested with 100 mite-infested plants. Infested plants were repeatedly transplanted into these border areas at 2-week intervals until the plants in the plots were 2 months old. Although immediate spraying was planned following introduction of the mites, wind velocities made spraying impractical for 11 days. Counts were made as in the spring field tests after 5 and 10 weeks on 10 terminal leaves taken at random from different locations within each plot.

Initial seed treatments[5] were applied in the greenhouse to Pawnee wheat, using systemic insecticides blended with various inert carriers. Germination tests consisted of (1) planting in soil flats immediately after treatment and recording both reduced and retarded plant emergence; and (2) Kansas State Seed Laboratory, Topeka, tests immediately after treatment and also after 30 days' storage and based upon percentage of germinating seeds after 1 week.

Application of the toxicant-carrier formulation to seed as outlined by Kantack (1955) was started at a relatively high rate of 1.5, 1, and 0.5 pounds of toxicant per bushel of seed. Because of resulting low germination, the amount of toxicant was reduced at regular increments until germination surpassed 80% in the highest level of toxicant used, as compared with 92% germination in the untreated seed. Six treated wheat seeds were planted in in each of five 5-inch flower pots for each formulation.

A water emulsion soil drench was simultaneously employed in the greenhouse as soon as the wheat seedlings began to emerge, using the same rates of toxicant per acre as in the seed treatments. It was applied adjacent to the plants when the soil was fairly dry.

Mite infestation for both seed treatments and soil drench was started when at least one plant had emerged in each pot. All plants in each pot were not infested at this time, however, because wheat plants infested before the 2-leaf stage in the greenhouse generally fail to grow well because of heavy mite damage. One seedling in each of the pots was infested with approximately 80 to 100 mites by cutting a $\frac{3}{4}$-inch piece of infested, curled, wheat leaf and placing it in the first uncurling leaf. In cases where the single leaf had not uncurled sufficiently to hold the cut section, it was placed vertically beside the plant. The initial 3-day counts on the first of two tests were made by removing the plants and counting the mites on the entire upper surface of the leaf. At this same time, mite infestations for the 2-week count were made on the second plant of each pot. After the second weekly count, no more plants were sacrified, and only the terminal leaves were removed for counts. In cases where less than five seeds per pot failed to germinate, additional seeds were added when the first count was made. In those pots where all six seeds had germinated, the plants were thinned to leave only four plants after the first mite count. After the second weekly count, all the plants remaining in the experiment were infested. Weekly counts and new mite infestations were continued and data and observations recorded for 7 weeks. As a means of randomizing the samples, pots were moved from place to place weekly by groups according to treatments.

Chemicals appearing most effective in the first experiment were retested in a second experiment, using twice the number of plants per treatment. This time all mite infestations were made by leaning the infested cut-leaf portion of the plant vertically against the wheat seedlings. The previous experiment had shown that successful mite transfer could be made in this manner. In addition, Am. Cyanamid 12008 and 12009 were impregnated on granules of 15–30 mesh attaclay. These granules were fixed temporarily for sowing purposes to the wheat seed with the same inert material, Carbowax 6000, used in preparation of the toxicant-carrier formulation. Controls consisted of untreated seed and seed treated with Carbowax 6000 and attaclay only.

For field tests, Pawnee wheat seed with a germination rate of 94% was treated with the three concentrations of each of six toxicant-carrier formulations, the highest being near the level which had previously been found to reduce germination. The plot was 45 feet wide by 350 feet long. Each treatment consisted of four rows of wheat approximately 36 feet long and replicated three times. One blank row was left between each treatment. Four rows of border wheat were seeded on all sides of the plot into which 175 mite- and mosaic-infested plants were transplanted every 2 weeks for 2 months to provide continuous infestations. Three hundred similar plants were transplanted between the rows within the treatments. Counts were based upon average number of mites on 30 centrally located leaves.

Phytotoxicity records were determined by visual appearance.

RESULTS AND DISCUSSION.—*Preliminary Greenhouse Screening.*—Although 30 chemicals and formulations were tried in the greenhouse, the only combinations of both high initial mite control and long residual effect were light mineral seal oil at 5 gallons per acre, Strobane at a high rate of 8 pounds, Diazinon at 1 pound, and lindane at 3 pounds actual toxicant per acre. However, all of these treatments injured the plants when used at these rates.

The organic phosphorous compounds, parathion at 0.25 pound, Phosdrin at 0.65 pound, and Diazinon at 0.25 pound toxicant per acre gave high initial reductions in populations, followed by increases after a few days. The chlorinated compounds, dieldrin at 1 pound, Chlorobenzilate 1 to 1.5 pounds, and endrin at 0.5 pound toxicant per acre, generally produced low initial population decreases with subsequent population reductions up to 28 days.

Spring Field Spray Tests.—Results are given in table

[5] Chlorthion lot no. 23-54-30, demeton lot no. 23-54-30/1, and methyl demeton lot no. 23-54-21, supplied by Pittsburg Coke and Chemical Co.; Am. Cyanamid 12008, 88%, and 12009, 88.3%, supplied by American Cyanamid Co.; schradan lot no. V-831, supplied by Monsanto Chemical Co.

Table 1.—Spring field-spray tests for control of *Aceria tulipae*, Manhattan, Kansas. 1954.

Treatment	Lbs./Acre	Per Cent Control After Days Indicated		
		2	10	28
		Sprayed April 27		
Check		(528 mites)	(279 mites)	(281 mites)
Chlorobenzilate	1.0	65	−1[a]	−34
Parathion-oil[b]		65	−11	78
Shell OS-1808	0.65	90	23	−70
Phosdrin	0.65	83	−109	60
Endrin	0.5	84	0	−23
Malathion	2.0	86	−50	40
NPD	1.0	84	−5	53
		Sprayed May 10		
		3	10	28
Check		(896 mites)	(514 mites)	(39) mites)
Am. Cyanamid 12009	0.5	88	−24	−92
Dieldrin	1.0	82	70	−8
Lindane	2.0	51	32	−8
Parathion	0.5	81	−79	−113
Chlordane	1.0	84	43	−67
Diazinon	0.5	52	−14	−26
Toxaphene	3.0	83	7	−144

[a] Negative numbers indicate percentage increase over check.
[b] Parathion @ 0.2 lb. per gallon in light mineral seal oil @ 3 gal./acre (lot No. LS-0289, Standard Oil Co., Indiana); caused slight burning and russeting of leaves and slight stunting.

1. During the 2- to 3-day period following spraying, control ranged from 90% (Shell OS-1808, 2-carbethoxy-1-methylvinyl diethyl phosphate) to 51% (lindane). With the exception of dieldrin and parathion at 10 days, all treatments gave low control, the mites often being more abundant in the treatments than in the checks. Heavy rains following spraying undoubtedly influenced the effectiveness, and this factor was considered when selections were made of sprays for the fall field spray tests.

Phytotoxicity observed was not as severe as in the greenhouse. Parathion in oil solution caused phytotoxicity in the jointing stage. In general, other toxicants which caused only slight to moderate phytotoxicity in the greenhouse, exhibited no symptoms of plant injury in the field.

Mite populations were located on the plants farther from the ground as the season progressed. This movement toward new plant tissues as the culms elongated resulted in many mites not remaining in contact with the sprayed portions. Although growth is not as extensive in fall wheat plants, more tillering takes place, which, along with the washing influence of rains, further indicates the advantage of systemic insecticides.

Fall Field Spray Tests.—Results are given in table 2. Lindane at 0.5 pound in 1 gallon of light oil per acre burned the young plants severely. Phytotoxicity was so severe that some of the plants failed to recover. Those which survived did so by forming new growth from the crown. Phytotoxicity of 0.5 pound of parathion in 1 gallon of light oil per acre, although not so severe as lindane-oil in retardation of plant growth, nevertheless, reduced above-ground portions of the plants. This reduced growth may partially account for the relatively low populations of mites on these treatments at the 5-week count.

Table 2.—Fall field-spray tests for control of *Aceria tulipae* on wheat plants subjected to continuous mite infestation. Manhattan, Kansas. 1954.

Treatment	Pounds per Acre	Per Cent Control After	
		Five Weeks	Ten Weeks
Untreated		(5 mites)	(13 mites)
Parathion	0.5	−40[a]	−77
Strobane	3.0	20	−23
Orthorix	2.0	20	23
Endrin	0.75	60	54
NPD	1.0	20	23
Lindane-oil	(b)	80	62
Chlorobenzilate	2.0	40	31
Dieldrin	1.0	40	0
Karathane	1.0	80	−8
Am. Cyanamid 12008	0.5	60	−62
Parathion-oil	(c)	60	92
Phosdrin		80	46
Endrin-oil	(d)	100	0
Schradan	2.0	40	−162

[a] Negative numbers indicate percentage increase over check.
[b] Lindane 0.5 lb. in 1 gallon light oil per acre, lot no. LS-0340, Standard Oil Co. (Indiana). Severe burning caused.
[c] Parathion 0.5 lb. in 1 gallon light oil per acre, lot no. LS-0337, Standard Oil Co. (Indiana). Caused burning.
[d] Endrin 0.4 lb. in 1 gallon light oil per acre, lot no. LS-0338, Standard Oil Co. (Indiana).

The 5-week counts indicated mites present on all treatments except where 0.4 pound endrin in 1 gallon of light oil per acre was applied. Phosdrin at 0.65 pound and Karathane at 1.0 pound per acre gave 80% control. After 10 weeks mite populations on treatments sprayed with organic phosphorous compounds, with the exception of parathion in oil, were not much reduced and sometimes greater than on checks. This apparent build-up was also evident in some of the spring spray tests. Best control without plant injury at 10 weeks was attained by endrin at 0.75 pound (54% control).

Seed Treatments and Germination Tests with Systemic

Table 3.—Germination of Pawnee wheat following various seed treatments.

Treatment[a]	Pounds per Bushel	Per Cent Reduction, Days after Treatment		
		Soil Flat Emergence Tests	7-Day Germination Test, (State Seed Testing Lab.)	
		12	1–2	30
Untreated[b]		(86% emergence)	(92–95% emergence)	(92% emergence)
Schradan	0.125	0	0	10
	0.5	6	0	18
Chlorthion	0.125	15[c]	6	23
	0.5	19	28	88
Methyl demeton	0.125	5	2	1
	0.5	4[c]	3	14
Demeton	0.125	4[c]	5	2
	0.5	5[c]	9	25
Am. Cyanamid 12008	0.125	0	4	4
	0.5	12[c]	4	11
Am. Cyanamid 12009	0.125	1	6	9
	0.5	4[c]	11	8

[a] Carrier was 4 pounds Carbowax 6000 per bushel, except portion of untreated checks.
[b] No significant difference between untreated seed and Carbowax 6000-treated seed @ 4 lbs./bu.
[c] Retarded emergence.

Toxicants.—Data on germination are presented in table 3. Retardation and/or reduction occurred in the case of all formulations, although not above the 5% confidence level in the case of methyl demeton, demeton, and Am. Cyanamid 12008 at the 0.125 pound per bushel rate.

Results of mite control by seed treatments in the greenhouse are given in table 4. Untreated plants did not differ in appearance from the treated plants. Best control at the end of 1 week was attained by Am. Cyanamid 12009 as a regular seed treatment (95%) and in granulated form (89%). Am. Cyanamid 12008 ranked second as a regular seed treatment (85%) and in granulated form (74%). Both of these compounds retained a control of 50% or above through 5 weeks as a regular seed treatment and 98 and 97% in granulated form. These compounds were also the most effective at the end of 7 weeks.

The maximum effectiveness of schradan was from the second through the fifth week; for Chlorthion the first week; for methyl demeton the second week in the case of soil drench; and for demeton the second through fifth week.

In general, seed treatment produced a higher per cent control than soil drench, the difference being most apparent during the first week. This was largely attributable to a delay of approximately 3 days in applying the drench to avoid possible effects on the germinating seed. Another possible reason for initially higher mite counts may have been that treating the soil by drench resulted in the mites moving more rapidly from the cut leaf used for infestation, thus, infesting the host plant sooner.

Table 4.—Control of *Aceria tulipae* by seed treatments and soil drench in the greenhouse. Manhattan, Kansas.

Treatment	Weeks Following Treatment	Per Cent Control	
		Seed Treatment	Soil Drench
Check	1	38 mites per plant	
	2–3	80 mites per plant	
	4–5	74 mites per plant	
	6–7	140 mites per plant	
Am. Cyanamid:			
12008-carbowax-granules	1	74	—
	2–3	98	—
	4–5	97	—
	6–7	61	—
12009-carbowax-granules	1	89	—
	2–3	82	—
	4–5	98	—
	6–7	79	—
12008[a]	1	85	89
	2–3	70	66
	4–5	50	41
	6–7	54	40
12009[a]	1	95	37
	2–3	55	54
	4–5	54	31
	6–7	27	28
Schradan[a]	1	9	−59[b]
	2–3	53	32
	4–5	32	−6
	6–7	−37	−121
Chlorthion[c]	1	55	26
	2–3	17	18
	4–5	3	20
	6–7	−7	21
Methyl demeton[a]	1	−39	−7
	2–3	31	54
	4–5	33	30
	6–7	15	−6
Demeton[a]	1	15	−142
	2–3	40	44
	4–5	42	52
	6–7	−34	24

[a] Treatment rates were an average of 0.125, 0.25 and 0.5 lb. per bushel.
[b] Negative numbers indicate percentage increase over check.
[c] Chlorthion rates were an average of 0.067, 0.125 and 0.25 lb. per bushel.

Table 5.—Control of *Aceria tulipae* following seed treatment in the field.[a] Manhattan, Kansas.

Treatment[b]	Per Cent Control After		
	Five Weeks (24 mites)	Ten Weeks (10 mites)	April 5, 1955 (9 mites)
Untreated			
Am. Cyanamid:			
12008-carbowax granules	58	−10[c]	−110
12009-carbowax granules	62	−150	0
12008	89	−10	0
12009	72	−27	−90
Schradan	76	13	52
Chlorthion	84	−17	86
Methyl demeton	77	3	−110
Demeton	84	−53	−44

[a] Planted September 23, 1954.
[b] Same dosages as given in table 4.
[c] Negative numbers indicate percentage increase over check.

Results of field tests with treated seed are given in table 5. Control was generally better than in the greenhouse tests at the end of 5 weeks, probably because of higher infestation rates in the greenhouse; the exceptions were Am. Cyanamid 12008 and 12009 impregnated in granules. At the end of 10 weeks, treatments were generally more heavily mite-infested than the checks. Counts during the following spring showed erratic results. In all instances the small numbers of mites made accurate control percentages unlikely but they did show that at no time were plants mite-free.

Viruliferous mites were constantly liberated in enormous numbers onto the field plots and wheat streak mosaic, determined by Webster H. Sill, Jr., and Hurley Fellows of the Department of Botany and Plant Pathology, Kansas State College, developed in the great majority of replications within 1 to 2 feet of the area where mite liberation occurred. There was no difference in wheat streak mosaic symptoms among the various treatments or the controls. It is apparent that the degree of mite control attained under these unusually severe conditions was not sufficient to reduce the disease incidence, even though percentage of control of mites was relatively high shortly after fall planting which is generally considered the most critical period of infection (Gibson *et al.* 1954). Seed treatment of large areas with the most promising of the chemicals might kill sufficient numbers of newly-arrived mites before reproduction, thus reducing dispersal of potentially viruliferous offspring. Since mites arriving from a distance are less numerous than the offspring that could subsequently build up locally, treatment of a large area early in the fall might reduce the percentage of plants with mosaic although this has not been proved. Limitations of the use of sprays for mite control, as feasible for disease control, are discussed by Staples & Allington (1956).

REFERENCES CITED

Fellows, H. 1949. A survey of the wheat mosaic disease in western Kansas. Plant Dis. Reporter. **33**: 356–8.

Gibson, William W., W. H. Sill, Jr., C. L. King and Dell E. Gates. 1954. Important questions on wheat streak-mosaic. Cargill Corp. Bull. **29**(6): 21–22.

Kantack, Edmon J. 1955. Carbowax as a carrier of systemic insecticides for treating wheat seed. Jour. Econ. Ent. **48**(4): 486.

Kantack, Edmon J., and Herbert Knutson. 1954. Location of maximum numbers of the wheat curl mite, *Aceria tulipae* (K.) (Eriophyidae) on the winter wheat plant during spring growth. Field and Laboratory 22(3): 65–67.

Melchers, L. E. 1950. The 1949 outbreak of wheat mosaic. Bien. Rpt. Kansas State Bd. Agric. **37.**

Sill, W. H., Jr., Hurley Fellows and Claude L. King. 1955. Kansas wheat mosaic situation (1953–54). Plant Dis. Reporter. **39**(1): 29–30.

Slykhuis, J. T. 1953. Wheat streak mosaic in Alberta and factors related to its spread. 1953. Canadian Jour. Agric. Sci. **33**: 195–7.

Staples, R., and W. B. Allington. 1956. Streak mosaic of wheat in Nebraska and its control. Nebraska Agric. Expt. Sta. Res. Bull. **178.**

The Weight Responses of Beef Cattle in Relation to Control of Horn and Stable Flies[1]

L. K. CUTKOMP and A. L. HARVEY, *Departments of Entomology and Economic Zoology and Animal Husbandry, University of Minnesota, St. Paul*

ABSTRACT

Repellent-insecticide oil formulations were tested in a treadle sprayer for the control of biting flies (predominantly *Siphona irritans* (L.) and *Stomoxys calcitrans* (L.)) on grazing beef cattle (weighing from 600 to slightly over 900 pounds) for three summers at the Rosemount Agricultural Experiment Station of the University of Minnesota, 1954–56, with the following results: (1) In 1954 and 1955, significant weight gains were realized on cattle which had been treated to reduce the number of biting flies. The lower gain in weight in the treated cattle in 1956 may have been due to cool temperatures, abundant rainfall and a resultant excellent forage crop. (2) Control of horn flies exceeded 95% and reduction in numbers of stable flies was about 70%. (3) Pyrethrins plus piperonyl butoxide or MGK-264 gave satisfactory fly control, but the addition of butoxy polypropylene glycol to the formulation permitted a reduction in quantity of pyrethrins content without loss of efficiency. (4) Individual animals, within a breed, had greater variations in numbers of biting flies than occurred between breeds. (5) On the basis of these results, and a study of weather records, a profitable return from biting fly control may be expected in most years.

During three summers (1954–56) comparisons have been made of weight changes on sprayed and unsprayed beef cattle. The fly sprays have been applied with two-nozzle automatic treadle sprayers. The biting flies were primarily two species, the horn fly, *Siphona irritans* (L.), and the stable fly, *Stomoxys calcitrans* (L.). A few horse flies, *Tabanus* sp., were observed early in each season but were of little significance during any of the seasons. The studies were carried out at the Rosemount Agricultural Experiment Station, Rosemount, Minnesota.[2]

Ideally the influence of biting fly populations on weight responses of cattle should be made on infested lots of animals in contrast to animals devoid of biting flies. This was not quite accomplished but was nearly achieved against horn flies (over 95% control). Control of the stable flies was about 70 to 75%. Thus, some influence of the biting flies remained. However, the experiments were designed to provide information about the value of a reasonably high level of practical biting fly control measures.

PROCEDURE.—Each year four beef cattle were placed in a lot, whether treated or untreated. The lots were equal areas of a common pasture divided by an electrified barbed wire fence. Cattle in two lots were treated in each of the first 2 years and one group left untreated. In 1956 only one group was treated, with one group left untreated. The cattle were matched so that equal numbers of the same breed and sex occurred in each group. Weights were taken at the start of the experiment, June 1 to 15. The experiments were terminated between September 1 and 15 each year.

Biting fly counts were made from one to three times per week at mid-day periods, between 10:30 A.M. and 2:30 P.M. Counts were made on standing animals, and when necessary, field glasses were used.

MATERIALS.—All spraying was done with a two-nozzle treadle sprayer equipped with a metal canopy and canvas flaps to reduce the effect of the wind.[3] An upper nozzle was located to spray over the back, a lower nozzle directed the spray to the region of the lower legs.

The spray mixtures used continuously throughout each season for comparisons of weight changes in the sprayed and unsprayed groups of beef cattle were as follows: first, for 1954, 0.54% pyrethrins, 5.4% MGK-264, 10% emulsifier, and 53.29% petroleum distillate; for

[1] Paper No. 3809 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1. Accepted for publication August 22, 1957.

[2] The close cooperation of members of the Departments of Animal Husbandry, Soils, and Agronomy & Plant Genetics has made this study possible.

[3] Banafly Automatic Sprayers, American Scientific Laboratories, Madison, Wisconsin. Acknowledgement is made to the American Scientific Laboratories and McLaughlin Gormley King Co. for making the sprayers available. Chemicals were provided by the latter company and also by Union Carbide Chemicals Co., White Plains, N. Y. Fairfield Chem. Div., Food Machinery and Chem. Corp., Baltimore, Md., Hercules Powder Co., Wilmington, Delaware, and Rohm and Haas Co., Philadelphia, Pa.

1955 and 1956 a second mixture known as AT-4 was used in which the pyrethrins were reduced to 0.23% plus 2.26% piperonyl butoxide, and 55.45% butoxy polypropylene glycol.

The effectiveness of other oil based sprays, particularly with respect to reduction of biting flies, was evaluated. In 1954 these sprays included (a) Pyrocide concentrate containing 1% pyrethrins, 10% MGK-264 with emulsifier in petroleum distillate, and (b) 0.5% pyrethrins, 5.0% MGK-264, 1% MGK Repellent 11 (2, 3, 4, 5-bis (Δ_2 butylene) tetrahydrofurfural) with emulsifier in petroleum distillate. They were compared in early summer, June 17 through August 9. Two additional materials used from August 9 through September 14, 1954 were (c) 15% Lethane 384 plus 5% Perthane in deodorized kerosene and (d) 10% MGK Repellent 326 in deodorized kerosene. Repellent 326 (d) was also tried in 1955 but apparent decomposition prevented obtaining an adequate evaluation. The usefulness of this repellent has been reported elsewhere (Bruce 1956).

In 1955 comparative spray materials were (e) Pyrenone (0.5% pyrethrins plus 5% piperonyl butoxide) used from June 15 to August 7 and (f) 7% Thanite and (g) 5% Thanite plus butoxy polypropylene glycol. Both (f) and (g) were used from August 7 to September 13, 1955.

RESULTS.—Sprayed beef animals weighing an average of 904 pounds at the start of the 1954 experiment had increased their average weight by 65 pounds to 969 pounds by September 14. Untreated cattle increased an average of 43 pounds from a 908 average to 950 pounds. These gains during the summer fly season are shown as 0.73 pounds gain per animal per day in table 1 for the treated group and 0.48 pounds for untreated animals. In 1955 both groups gained as in the previous year, but the differences were greater in favor of the treated group. The average weight of the treated group increased from 668 to 770 pounds and the untreated from 696 to 757 pounds. The difference in weight gains was increased when a fifth Hereford heifer was added to each lot on August 3. Each of the additional heifers weighed between 1000 and 1100 pounds. The gains of 1.3 pounds per day per treated animal shown in table 1 compare with 0.63 pounds per day per animal of the untreated during the 1955 biting fly season. There was no case where an untreated animal of the same breed gained as much as a treated animal of the same breed during the 1954 and 1955 seasons. The consistency of results in these 2 years was sufficient to provide significant differences in weight changes when subjected to analysis of variance.

In 1956 the treated animals did not gain as much as the untreated. One treated Shorthorn gained 44 pounds, more than any other animal in either group. Two of the sprayed animals did not differ more than 2 pounds at the beginning and end of the season. The sprayed group averaged 753 pounds at the start and 783 pounds at the end of the summer, an average gain of 30 pounds per head. The untreated group went from 745 to 804 pounds with an average gain per head of 59 pounds during the season. Because of the variability of weight changes between different animals the two groups did not differ to a significant degree at the 5% level.

Weather records were taken each year (table 3) and the relevancy to weight gains is presented in the discussion.

Table 1.—Comparisons of reductions in populations of biting flies, and of weight changes on four beef cattle per treatment.[a]

MATERIAL	NO. OF PRINCIPAL BITING FLIES PER ANIMAL		POUNDS GAINED PER ANIMAL PER DAY	EXPERIMENTAL PERIOD
	Horn Flies	Stable Flies		
		—1954—		
0.5% Pyrethrins+5% MGK-264+BPG[b]	0.3	4.7	0.73[c]	June 16–Sept. 14
1.0% Pyrethrins+10% MGK-264	0.3	6.6	0.28	June 16–Aug. 9
Untreated	58.0	13.3	−0.01	June 16–Aug. 9
Untreated	46.4	10.7	0.48	June 16–Sept. 14
		—1955—		
AT-4	1.6	6.1	1.30[d]	June 14–Sept. 13
Pyrenone	2.5	6.1	1.33	June 14–Aug. 3
Untreated	69.3	28.5	0.52	June 14–Aug. 3
Untreated	48.9	22.6	0.63	June 14–Sept. 13
		—1956—		
AT-4	7.3	8.1	0.36[e]	June 20–Sept. 10
Untreated	98.0	23.0	0.72	June 20–Sept. 11

[a] Insecticides and repellents were used in treadle sprayers for at least 50 consecutive days during each of the three fly seasons.
[b] Butoxy polypropylene glycol, also known as Cray Fly Repellent.
[c] Wt. gain significantly greater than untreated at 5% level in 1954.
[d] Wt. gain significantly greater than untreated at 1% level in 1955.
[e] Difference not significant in 1956.

Weight differences for a 53-and 50-day period in 1954 and 1955, respectively, are also shown for two other pyrethrins formulations listed as (a) and (b) under materials. They were about equal in effectiveness on biting flies but were not used throughout the season.

Nearly all the spray materials compared (tables 1 and 2) gave a 95% reduction of horn flies in each year of study. This was on the basis of counts on untreated animals which averaged 46 (in 1954), 49 (in 1955) to 98 (in 1956) horn flies per animal during a mid-day counting period. Considerable seasonal fluctuation in numbers occurs on untreated animals (fig. 1).

Incidental to the major objective of the study, that pertaining to changes in weights of the animals, the several spray materials were compared and found to differ greatest in the reduction of numbers of stable flies. The

Table 2.—Comparisons of reductions in populations of biting flies with repellents and insecticides which were not used for a sufficient period to give reliable comparisons of the weights of the animals under test.

MATERIAL	NUMBER OF FLIES PER ANIMAL PER OBSERVATION				EXPERIMENTAL PERIOD
	Horn Flies		Stable Flies		
	Number	Per Cent Reduction	Number	Per Cent Reduction	
		1954			
Lethane-Perthane 0.5% Pyrethrins+5% MGK-264+BPG[a]	0.3	98.1	1.3	69.0	Aug. 9–Sept. 14
	0.1	99.4	1.4	66.7	Aug. 9–Sept. 14
Untreated	16	—	4.2	—	Aug. 9–Sept. 14
0.5% Pyrethrins+R-11	1.2	97.9	7.0	47.4	June 16–Aug. 9
Untreated	58.0	—	13.3	—	June 16–Aug. 9
		1955			
Thanite	2.1	94.8	11.9	39.5	Aug. 3–Sept. 13
Thanite+BPG	0.1	99.8	9.8	41.0	Aug. 3–Sept. 13
0.5% Pyrethrins+5% MGK-264+BPG	0.7	98.2	5.4	67.3	Aug. 3–Sept. 13
Untreated	40.0	—	16.6	—	Aug. 3–Sept. 13

[a] Butoxy polypropylene glycol.

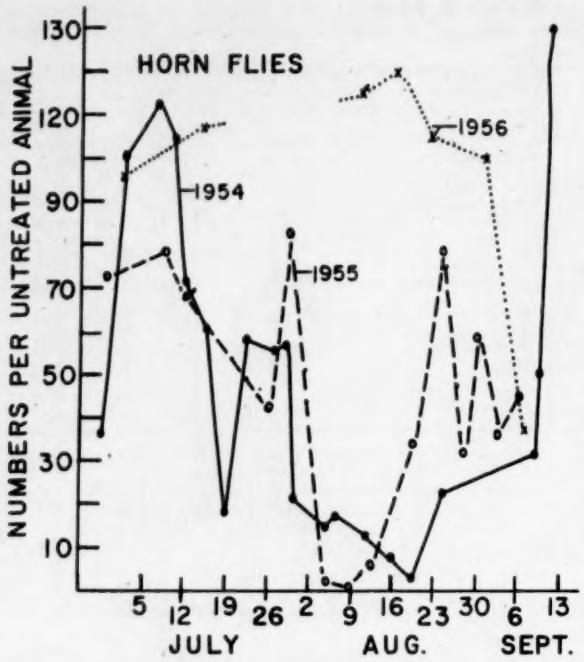


FIG. 1.—The numbers of horn flies per untreated grazing beef animal at each observation during three consecutive summers at Rosemount, Minn. Each count is the mean number on four animals.

pyrethrins sprays used in 1955 as shown in table 1 were effective in reducing stable flies by 73 % (22.6 flies in untreated reduced to 6.1 flies per animal per observation). Some spray combinations gave only 40 or 41% reduction in numbers of stable flies (table 2).

The possible influence of breeds of beef cattle on numbers of biting flies was examined. Comparisons are shown among untreated beef cattle in table 4. Actually, the mean number of flies per animal per observation is rather close considering the day to day variability. The horn fly and stable fly variation within a breed was high. Averages of horn flies were 46.9 and 66.4 on two different Shorthorn heifers in 1954 and average stable fly counts were 22.5 and 29.0 on two different Hereford steers in 1955. Actually, individual variation per animal was greater than differences between the breeds used in the experiment.

DISCUSSION.—The results give further evidence that the automatic treadle sprayer is a useful piece of equipment for reducing the numbers of biting flies on grazing cattle which are not housed during the summer. In this equipment horn fly control was excellent and stable fly control moderately good. The treadle sprayer was only useful where a water supply or similar strong inducement caused the cattle to use the sprayer at least once per day.

The results indicate that the use of a repellent-insecticide mixture which gives good biting fly control may result in measureable weight gains. Nevertheless, expected weight gains may not occur under some circumstances. They did not occur in 1956, and the comparative gains of 1955 were of greater magnitude than in 1954. The explanation for these yearly fluctuations does not appear to lie in the degree of fly control nor in any marked differences in intensity of biting fly attack which would correspond to the seasonal weight changes. There is no detectable influence of age, size or breeds of animals.

Observations showed that green, succulent forage was available throughout the summer of 1956, while the pasture became dry in late summer of 1954 and even drier during the 1955 season. No food supplements were given in either of the three seasons. The forage conditions are related to seasonal temperature and rainfall (see table 3). The Rosemount records reveal that 1956 was a cool (daily mean temperature of 65.2° F.), wet summer, a total of 13.64 inches of rain fell during the experimental period. The average daily temperatures were 68.2° F. and 70.7° F. in 1954 and 1955, respectively, with total rainfall somewhat less than 11 inches. The number of days having maximum daily temperatures 85° F. and above were 14. 40 and 10 for the respective years of 1954, 1955, and 1956. St. Paul weather records show that there was no time in the last 40 years when the July-August rainfall exceeded that of 1956. Further, only 2 years (1950 and 1951) since 1915 had cooler daily average temperatures in these summer months.

Physiological stresses do occur on grazing animals subjected to high temperatures and moderately or extremely

Table 4.—A comparison of horn fly and stable fly abundance on different breeds of beef cattle not treated with an insecticide or repellent.[a]

BREED	SEX	NUMBER OF OBSERVATIONS	MEAN NO. BITING FLIES PER OBSERVATION		
			Horn Flies	Stable Flies	Year
Hereford	Heifer	9	35.4±41.8	8.9± 5.4	1954
Shorthorn	Heifer	19	46.9±42.8	11.5± 7.2	1954
Shorthorn	Heifer	19	66.4±43.7	10.5± 5.5	1954
Red Poll	Heifer	19	49.3±44.1	10.6± 5.9	1954
Hereford	Steer	10	49.5±39.5	29.0±21.9	1955
Hereford	Steer	10	46.8±37.9	22.5±15.7	1955
Red Poll	Heifer	10	45.6±39.1	26.4±15.2	1955
Red Poll	Heifer	10	54.8±44.9	24.2±11.3	1955

[a] Numbers of flies per observation are given with the standard deviation. There were no significant differences due to breeds or sex of animals.

Table 3.—A comparison of temperature and rainfall records during the summers of 1954–56 at Rosemount, Minnesota.

PERIODS	AVERAGE DAILY MAXIMUM			AVERAGE DAILY MEAN			NO. DAYS 85° AND ABOVE			INCHES OF RAINFALL		
	1954	1955	1956	1954	1955	1956	1954	1955	1956	1954	1955	1956
June 20–30	81.5	81.0	80.5	71.6	69.1	68.9	1	1	4	1.66	0.23	2.35
July 1–31	82.0	86.9	77.4	70.9	76.0	66.4	10	21	2	2.85	5.84	4.90
Aug. 1–31	79.4	81.7	79.3	67.8	69.1	67.5	3	17	4	3.48	4.62	5.87
Sept. 1–15	70.3	76.7	68.3	61.0	61.1	55.6	0	1	0	2.83	0.25	0.52
Average or Total	79.0	82.6	76.9	68.2	70.7	65.2	14	40	10	10.82	10.94	13.64

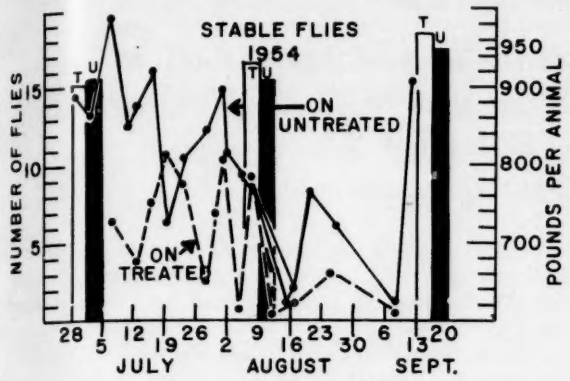


FIG. 2.—The numbers of stable flies on treadle-sprayed and unsprayed grazing beef animals recorded during the summer of 1954 at Rosemount, Minn. Changes in average weights of the four beef animals are shown as histograms. The solid bar (U) indicates unsprayed animals, the open bar (T) treated animals. The reduction in numbers of horn flies (table 1) must also be considered as influencing weights.

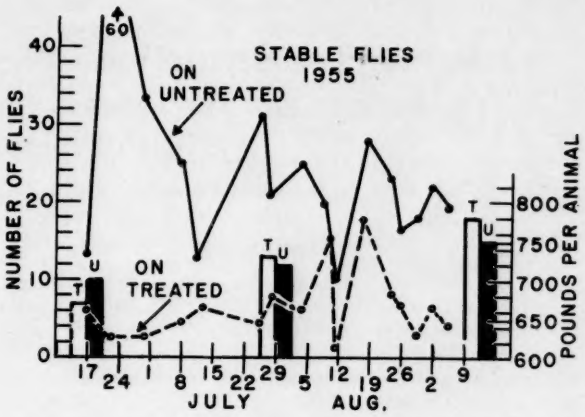


FIG. 3.—The numbers of stable flies on treadle-sprayed and unsprayed beef grazing animals recorded during the summer of 1955 at Rosemount, Minn. Changes in average weights of the four beef animals are shown as histograms. The solid bar (U) indicates unsprayed animals, the open bar (T) treated animals. The reduction in numbers of horn flies (table 1) must also be considered as influencing weights.

dry summer conditions. The additional stress of biting flies attacking would appear to be greatest when these environmental conditions are combined with the need to obtain good forage. During these periods of stress the reduction of biting flies should be of greatest benefit to the animal. The additional influence of excessive rainfall washing off some of the spray material may have reduced the effectiveness somewhat. However, since the animals were sprayed one or more times per day this effect should not have been great, and fly counts were similar to other seasons.

The economic aspects of the weight increases which occurred in 1954 and 1955 deserve discussion. In 1954 the treated animals gained $\frac{1}{4}$ lb. per day more than the untreated, a value which would amount to 22.5 lbs. for a 90-day summer period. Assuming a price of 20 cents per pound this would amount to a $4.50 increase per head. In 1955, for a similar period 60.3 additional pounds would have resulted in an estimated increase in value of $12.06 per head. The cost of the spray material applied (assuming an $8 retail cost per gallon and four applications per day using 1.5 ml. of spray each trip) would be $1.14 for the 90-day period, or slightly less than 9 cents per week per animal. Thus a profit of $3.36 and $10.92 per head was realized in 1954 and 1955, respectively, and none in 1956. Original equipment cost and the small amount of labor required is not included in the estimate. Weight gains resulting from the use of DDT in controlling horn flies have been reported from Kansas (Laake 1946). They ranged from 30 to 70 lbs. per animal for at least a 4-month summer period. For comparative cost figures of other methods of biting fly control on animals, see Haynes & McKenzie (1954).

REFERENCES CITED

Bruce, W. G. 1939. Some observations on insect edaphology. Jour. Kansas Ent. Soc. **12**(3): 91–93.

Bruce, W. N. 1952. Automatic sprayer for control of biting flies on cattle. Illinois Nat. Hist. Survey Biol. Notes No. 27.

Bruce, W. N. 1956. Are fly repellents practical? Pest Control **24**: 14, 58.

Granett, Philip, E. J. Hansens, and C. T. O'Connor. 1955. Automatic cattle sprayers for fly control in New Jersey. Jour. Econ. Ent. **48**: 386–9.

Haynes, H. L., and R. E. McKenzie. 1954. Evaluation of methods for control of flies on livestock. Proc. 41st Ann. Meeting Chem. Spec. Mfrs. Assn. pp. 113–117.

Laake, E. W. 1946. DDT for the control of the horn fly in Kansas. Jour. Econ. Ent. **39**: 65–68.

NOTICE TO AUTHORS

Each manuscript submitted for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY must be accompanied by an abstract suitable for publication, and prepared in accordance with instructions for the preparation of abstracts as required by *Biological Abstracts*. This abstract will replace the usual author's summary. See "Important Notice to Authors" which appeared in the *Bulletin of the Entomological Society of America* 3(2): 1. June, 1957.

Relative Toxicity to House Flies of the *cis* and *trans* Fractions of Allethrin and the Intensity of Synergism of Their Mixtures with Piperonyl Butoxide[1]

W. A. Gersdorff and P. G. Piquett, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

Abstract

In a study with a new authentic sample of *cis*-allethrin, its toxicity relative to *trans*-allethrin and allethrin itself and the intensity of synergism of their 1:10 mixtures with piperonyl butoxide were evaluated when applied as kerosene sprays on the Campbell turntable. The house fly (*Musca domestica* L.) was used as the test insect.

cis-Allethrin was 0.546 as toxic as allethrin. The new estimation of the ratio for *trans*-allethrin and two previous estimations furnished a mean of 1.192 for that fraction.

On the basis of these two evaluations the *trans* fraction comprised 70% and the *cis* fraction 30% of the sample of allethrin. This result agrees very well with reported results obtained by an infrared spectrophotometric method.

On the basis of equivalents the piperonyl butoxide mixtures of *trans*-allethrin, *cis*-allethrin, and allethrin were, respectively, 2.94, 1.33, and 2.55 as toxic as allethrin alone.

Synergism was demonstrated in all three mixtures, its intensity ratio for each being about 2.6 at the 50%, 1.8 at the 5%, and 3.7 at the 95% mortality levels.

All sprays caused more than 90% knockdown of flies.

Allethrin is a mixture of esters of *dl*-allethrolone with *dl-cis-* and *dl-trans*-chrysanthemumic acids. There are eight optically active stereoisomers possible, four having a *cis* acid component and four a *trans* acid component.

In 1952 Gersdorff and Mitlin reported that, according to a bioassay with house flies (*Musca domestica* L.), the *cis* isomers comprised about 69% and the *trans* isomers about 31% of the distilled sample of allethrin used in their study. Reporting a determination made by L. Crombie by an infrared spectrophotometric method, Elliott (1954) stated that the sample of allethrin used in his bioassays contained 75% *trans* and 25% *cis* isomers. By a similar method Freeman (1955) found a fairly constant 80% *trans* fraction in 12 commercial samples of allethrin, and he concluded that there is little variation in the *cis-trans* proportion when allethrin is prepared according to the synthesis of Schechter *et al.* (1949).

The cause of this discrepancy between the results of the chemical and biological methods of assay, an almost complete reversal, has been the subject of much study. The problem has now been resolved. The bioassay depended on the estimations for the relative toxicity of the two fractions. These estimations were obtained on the first preparations of the materials, made in 1950 by Schechter *et al.* (1951). Subsequent chemical studies (LaForge *et al.* 1956) indicated that, although the *trans* fraction was authentic, serious doubts existed as to the purity of the *cis* fraction, at least some of it probably having been converted to the *trans* form during its preparation. With the advantage of this knowledge, which permitted them to avoid the conversion, in 1956 these chemists prepared a new sample of the *cis* fraction. The present paper reports the results of insecticidal tests made with this sample, a sample of the *trans* fraction, and their mixtures with piperonyl butoxide in comparison with allethrin alone and in similar mixtures. When a general term is required for brevity, allethrin and these fractions will be referred to as pyrethroids.

Materials.—The sample of allethrin used was a purified one, analyzing 93.4% by the hydrogenolysis method.

The sample of piperonyl butoxide used was the technical product obtainable commercially. Its relative toxicity as estimated by the same method (0.0106 as toxic as allethrin) has been reported (Gersdorff *et al.* 1957).

Procedure.—Sprays were prepared by dissolving the materials in refined kerosene (Deobase) and, after preliminary tests, diluting with the same solvent to selected concentrations on the sample basis. The mixtures with piperonyl butoxide were prepared only in the proportion of 10 times as much of that material as pyrethroid.

Knockdown and mortality were determined by the Campbell turntable method on house flies of the National Association of Insecticide and Disinfectant Manufacturers 1948 strain. All sprays were tested simultaneously and but once on each of six populations of flies. Approximately 105 flies were used in each test. The average age of the flies was 2 to 3 days.

To evaluate relative toxicity and determine the precision of the estimates, the mortality data were subjected to probit analysis as described by Finney (1952).

Mortality and Knockdown.—The mortality and knockdown data are summarized in table 1.

Parallelism of Regression Lines and Required Heterogeneity Factors.—Provisional individual regression lines were fitted graphically to the mortality data in table 1. As in previous studies with related materials, it appeared that the data could be adequately represented by two sets of parallel lines, those for the piperonyl butoxide mixtures being steeper than those for the pyrethroids alone. The statistical procedure based on this hypothesis was followed to obtain the weighted regression equations, and subsequent analysis of chi-squares gave no evidence that the data could not be so represented. However, heterogeneity factors were required in the calculation of error estimates; they were 7.549 for the pyrethroids alone and 10.270 for their mixtures.

Evaluation of Relative Toxicity and Intensity of Synergism.—The final equations showing the regression of mortality, expressed in probits, on concentration in milligrams per deciliter, expressed as logarithms, are given below. Those for the piperonyl butoxide mixtures are given on two bases, the expression of concentration in terms of pyrethroid content only (with constant 1) and of pyrethroid equivalents (constant 2). The standard

[1] Accepted for publication August 22, 1957.

Table 1.—Mortality and knockdown of house flies caused by kerosene sprays containing allethrin and its *cis* and *trans* fractions alone and mixed with piperonyl butoxide.[a]

Material	Concentration (Mg. per Dl.) Pyrethroid	Piperonyl Butoxide	Pyrethroid Equivalent[b]	Knockdown in 25 Minutes (Per Cent)	Mortality in 1 Day (Per Cent)
trans-Allethrin	200.0	—	—	100	94.8
	133.3	—	—	100	89.0
	88.89	—	—	100	80.1
	59.26	—	—	100	52.4
	39.51	—	—	100	37.5
	26.34	—	—	100	17.1
cis-Allethrin	300.0	—	—	100	96.1
	200.0	—	—	100	85.4
	133.3	—	—	100	68.2
	88.89	—	—	100	39.0
	59.26	—	—	99.5	14.5
	39.51	—	—	99.0	3.9
Allethrin	214.1	—	—	100	94.2
	142.8	—	—	100	90.4
	95.17	—	—	100	84.9
	63.44	—	—	100	60.2
	42.30	—	—	100	32.5
	28.20	—	—	99.7	10.4
Piperonyl butoxide plus—					
trans-allethrin	40.00	400.0	43.93	100	95.7
	26.67	266.7	29.29	99.9	81.0
	17.78	177.8	19.53	99.3	57.7
	11.85	118.5	13.02	96.0	12.9
	7.901	79.01	8.678	91.5	2.8
cis-allethrin	90.00	900.0	107.5	100	96.5
	60.00	600.0	71.65	100	86.1
	40.00	400.0	47.77	99.7	65.0
	26.67	266.7	31.85	97.1	20.4
	17.78	177.8	21.23	92.1	6.2
allethrin	42.30	423.0	46.78	99.7	94.5
	28.20	282.0	31.19	100	73.8
	18.80	188.0	20.79	97.7	36.0
	12.53	125.3	13.86	97.4	21.0
	8.355	83.55	9.241	93.1	3.3

[a] Six replicates.

[b] The pyrethroid equivalents of piperonyl butoxide are 0.009833 for the *trans* fraction, 0.01942 for the *cis* fraction, and 0.01060 for allethrin.

errors (s_b) of the generalized regression coefficients are also given.

	(1)	(2)	s_b
trans-Allethrin	$Y=3.4236\,X-0.9161$	—	0.150
cis-Allethrin	$Y=3.4236\,X-1.9289$	—	
Allethrin	$Y=3.4236\,X-1.0277$	—	
Piperonyl butoxide plus—			
trans-Allethrin	$Y=5.0425\,X-1.3128$	-1.5181	0.263
cis-Allethrin	$Y=5.0425\,X-2.8650$	-3.2538	
Allethrin	$Y=5.0425\,X-1.6117$	-1.8326	

From these equations LC-50's were estimated and relative toxicities were calculated as the inverse ratio of the relevant pair of estimations on the pyrethroid equivalent basis. They are reported in table 2. Each pyrethroid equivalent of piperonyl butoxide was calculated by means of the toxicities of piperonyl butoxide (as reported in the study with the *trans* isomers, Gersdorff *et al.* 1957) and the pyrethroids relative to allethrin. These equivalents are given in table 1.

The ratio of the toxicity of a mixture to that of the pyrethroid alone will, when significantly greater than unity, furnish an estimate of the intensity of synergism. The ratios together with their standard errors are also given in table 2. The requirements to demonstrate synergism are properly made on the log-concentration scale; therefore, also given are the logarithms of the intensity ratios and the minimum logarithm required to demonstrate synergism. The evidence of synergism was pronounced in all three mixtures.

DISCUSSION OF RELATIVE TOXICITY OF FRACTIONS.—*trans*-Allethrin has been precisely bioassayed three times by this method, two of them reported (Gersdorff & Mitlin 1952, Gersdorff *et al.* 1957) and now in this study. The authenticity of the samples, as evidenced in the chemical phase of the work by Schechter *et al.* (1951) and by recent unpublished data, was corroborated in the biological phase with ratios of toxicity to allethrin of 1.334, 1.163, and 1.078, the unweighted mean being 1.192. In view of the experimental errors and the fact that the estimations were obtained on different populations of flies, there is no real difference between these ratios.

The first preparation of *cis*-allethrin was found to be 0.854 as toxic as allethrin (Gersdorff & Mitlin 1952); the second 0.546. If it is assumed that the first one was simply a mixture of *cis*-allethrin and *trans*-allethrin, these ratios will reveal by a calculation similar to the following one that it contained about 48% *trans*-allethrin.

The proportions of *cis*-allethrin and *trans*-allethrin in the sample of allethrin used may now be calculated as before (Gersdorff *et al.* 1952) on the basis of their relative toxicities. The sum of the insecticidal equivalents of the components of a mixture equals the equivalent of the mixture, stated as a general equation as follows:

$$p_c R_c + (1-p_c) R_t = R_m$$

Table 2.—Evaluation of relative toxicities of *cis* and *trans* fractions of allethrin and relative effects of piperonyl butoxide in mixtures with them.

Material	LC-50 (Mg. per Dl.) Original Basis[a]	Pyrethroid-Equivalent Basis	Allethrin-Equivalent Basis	Relative Standard Error of Original LC-50 (%)	Relative Toxicity of— Mixture on Pyrethroid-Equivalent Basis	Principal Toxicant Alone	Intensity of Synergism at 50% Mortality Level	Log of Intensity Ratio	Log Required to Demonstrate Synergism	Range of Minimum Intensity Ratio Between 5 and 95% Levels
trans-Allethrin	53.46	53.46	57.63	4.59	—	1.078 ± 0.069	—	—	—	—
cis-Allethrin	105.6	105.6	57.63	4.50	—	0.546 ± 0.035	—	—	—	—
Allethrin	57.63	57.63	57.63	4.52	—	1.0	—	—	—	—
Piperonyl butoxide plus—										
trans-allethrin	17.86+178.6	19.62	21.15	4.11	2.937 ± 0.218	1.078	2.72 ± 0.20	0.435	0.0633	1.9–3.9
cis-allethrin	36.29+362.9	43.34	23.65	4.10	1.330 ± 0.096	0.546	2.44 ± 0.18	.387	.0612	1.7–3.5
allethrin	20.47+204.7	22.64	22.64	4.15	2.545 ± 0.190	1.0	2.55 ± 0.19	.406	.0634	1.8–3.6

[a] For the compound expressions the first figure refers to the pyrethroid.

in which p_c is the proportion of *cis*-allethrin and R_c, R_t, and R_m are the respective toxicity ratios of *cis*-allethrin, *trans*-allethrin, and the mixture. If ratios relative to allethrin are to be used, the ratio estimated for the new sample of *cis*-allethrin and the mean of the three estimates for *trans*-allethrin may be substituted in the equation as follows:

$$p_c(0.546)+(1-p_c)1.192=1.0$$

Whence $p_c=0.297$ and $1-p_c=0.703$.

Thus according to this bioassay the *cis* fraction comprised about 30% and the *trans* fraction about 70% of the sample of allethrin used in this study. With this authentic sample of *cis*-allethrin, therefore, the results for *trans*-allethrin by the biological method ($70\pm8\%$) agree very well with those by the infrared method (75 to 80%). Had the ratio, 1.078, obtained in this study for the toxicity of *trans*-allethrin been used in the equation, the content of that fraction would have been estimated as 85%.

DISCUSSION OF SYNERGISTIC EFFECTS.—The mixtures conform to the general pattern found in the previous studies of the *trans* fraction and isomers in similar mixtures (Gersdorff *et al.* 1957), in that the regression lines for the mixtures are a little steeper than those for the pyrethroids alone. Therefore, the intensity of synergism will differ somewhat at different mortality levels. The ratios calculated from LC-50's and LC-95's are also given in table 2, those for the mixtures being determined directly from the equations based on equivalents. The range is not very great, about 2 to 4 for all three mixtures. Therefore, for practical purposes the value estimated at the 50% mortality level may be accepted as descriptive of this relationship. All three mixtures are nearly three times as toxic as the pyrethroids alone; both fractions are synergized to the same degree as allethrin.

REFERENCES CITED

Elliott, M. 1954. Allethrin. Jour. Sci. Food and Agric. 5(11): 505–14.

Finney, D. J. 1952. Probit analysis. 318 pp. Cambridge.

Freeman, Stanley K. 1955. Infrared spectrophotometric determination of allethrin. Analyt. Chem. **27**: 1268–74.

Gersdorff, W. A., and Norman Mitlin. 1952. A bioassay of some stereoisomeric constituents of allethrin. Jour. Washington Acad. Sci. **42** (10): 313–8.

Gersdorff, W. A., Norman Mitlin, and P. G. Piquett. 1957. The relative toxicity, synergistic activity, and knockdown effectiveness of mixtures of piperonyl butoxide with allethrin and its **trans** fraction and isomers as house fly sprays. Jour. Econ. Ent. **50**(2): 150–6.

LaForge, F. B., Nathan Green, and M. S. Schechter. 1956. Allethrin. Synthesis of four isomers of *cis*-allethrin. Jour. Organ. Chem. **21**: 455–6.

Schechter, Milton S., F. B. LaForge, A. Zimmerli, and J. M. Thomas. 1951. Crystalline allethrin isomer, Jour. Amer. Chem. Soc. **73**: 3541–2.

Schechter, Milton S., Nathan Green, and F. B. LaForge. 1949. Constituents of pyrethrum flowers. XXIII. Cinerolone and the synthesis of related cyclopentenolones. Jour. Amer. Chem. Soc. **71**: 3165–73.

Response of Tobacco- and Tomato-Hornworm Moths to Black Light[1]

J. M. STANLEY[2] and C. B. DOMINICK[3]

ABSTRACT

Mechanical- and suction-type traps were tested in three tobacco fields near Chatham, Va., to study the response of the tobacco-hornworm (*Protoparce sexta* (Johan.)) and the tomato-hornworm (*P. quinquemaculata* (Haw.)) moths to black light and to evaluate these devices as a means of controlling hornworms. During the 3-year study, an appreciable number of tobacco-hornworm moths were captured, but only 17.9% were females. An increase in the number of tomato-hornworm moths caught occurred each year, and an overall average of 46.1% of these were females. A heavy infestation of hornworms did not occur during any of the years in which the investigations were in progress. The percentage of plants damaged in the test fields in comparison with check fields in the same vicinity, in most cases, indicated a slight suppression of damage where traps were used; however, they did not give the desired control. On the basis of these studies, it appears that the use of traps of the two designs that are equipped with black-light lamps with output characteristics of those used in this study as attrahents cannot be considered as a practical means of controlling hornworms on tobacco.

Insect traps with lamps as attrahents for catching nocturnal insects have been used for many years. Considerable evolution has occurred in the radiation sources used. Candles and kerosene lamps, with their yellow flames, were the first sources of radiation. The acetylene flame, with its white light, evolved later, to be followed by incandescent, mercury-vapor, and fluorescent electric lamps. The fluorescent lamps used in the work covered by this report radiate a large portion of their energy in the near-ultraviolet, or black-light, region of the spectrum and are referred to as "black-light lamps."

Work dealing with the attraction of harmful lepidopterous insects to lamps radiating energy in or near the ultraviolet portion of the spectrum has been reported. In studies to determine protection for sweet corn from the European corn borer (*Pyrausta nubilalis* (Hbn.)), Taylor & Deay (1950) used electrocutor traps equipped with H-4 reflector mercury-vapor and 30-watt black-light lamps. They reported a reduction of 57% in the population at a distance of 153 feet and 69.5% at 87 feet from the lamps during the first generation. Stahl (1954) reported that the most efficient traps tested in work on the tobacco-hornworm (*Protoparce sexta* (Johan.)) and the tomato-hornworm (*P. quinquemaculata* (Haw.)) moths were equipped

[1] Accepted for publication August 23, 1957.

[2] Agricultural Engineer, Agricultural Engineering Research Division, Agricultural Research Service, U.S.D.A., Blacksburg, Virginia.

[3] Associate Entomologist, Virginia Agricultural Experiment Station, Tobacco Research Station, Chatham.

with black-light lamps as attrahents. Although Stahl's traps caught an appreciable number of moths, his studies indicated that the use of light traps had little effect on the abundance of and damage caused by hornworm larvae near the trap. Pfrimmer *et al.* (1955), in evaluating two types of traps equipped with black-light fluorescent lamps for the control of the pink bollworm (*Pectinophora gossypiella* (Saund.)), found that the infestation increased throughout the period the cotton was fruiting. Glick & Hollingsworth (1956) introduced pink-bollworm moths into a tightly caged, 1/20-acre plot of cotton in which was located an electrocutor trap equipped with two black-light fluorescent lamps. This trap did not control or prevent a buildup to 100% infestation.

The tobacco hornworm is probably the most important insect pest affecting the production of tobacco in the Southeast. Haeussler (1952) estimated the annual loss due to this insect as $84,073,000 in the Southern States. The tomato hornworm also attacks tobacco; however, it is considered of minor importance in most of the flue-cured tobacco areas. In Virginia there are normally two broods of larvae of both species during the tobacco growing season. The first brood of the tomato hornworm is relatively important, but generally few second-brood larvae of this species are found on tobacco (Dominick 1950, 1951).

In 1953, because of the increased interest in electric traps, it was decided to test some of the commerically available equipment developed from results of earlier investigation by U. S. Department of Agriculture research engineers. This was done to obtain further information on the response of the two species of hornworm moths to radiation from black-light lamps and to evaluate the traps as a means of control. The studies herein reported were conducted at each of three locations near Chatham, Va., during the tobacco growing seasons of 1954–56, inclusive.

EQUIPMENT.—Two types of omni-directional traps were used in this study. The first, called the mechanical trap, was of sheet-metal construction supplied with brackets for mounting on a pole. It was equipped with two 30-watt black-light fluorescent lamps mounted horizontally slightly above and inside each long edge of the trough section of the trap. In the center of the trough, between the two lamps, there was mounted a vertical panel extending above the trap opening. This panel was painted with a green fluorescent paint, and the remainder of the trap was painted with a dull-black enamel.

Baffles were set in the throat section below the trough of the trap. These were arranged so as to permit insects to pass down into a plastic mesh-bag container and to prevent them from escaping up through the throat and out of the trap. The lamps on this unit consumed such a small amount of electricity that it was considered more satisfactory to keep the lamps energized at all times rather than to provide a switching system.

The second trap was equipped with a fan and designated as a suction trap. Four 15-watt black-light fluorescent lamps were mounted vertically, one in the apex of each of the four quadrants formed by the sheet-metal baffles. Below these baffles, which were painted with a green fluorescent paint, there was mounted in the cylindrical section an 18-inch, 4-blade propeller-type fan driven by a ⅓-hp. electric motor rated at 1725 r.p.m. The insects that were attracted to the lamp radiation would be drawn down by the fan and forced into the mesh bag below. Time switches were used to energize the circuits to the lamps and fan motor at twilight and to deenergize them just after dawn each day.

By operating the suction-type traps as outlined above, it was necessary to provide some means of preventing the escape of those moths not killed by the impact of the fan when they entered the trap. Two arrangements for doing this were utilized, the more satisfactory one of which was a deep cone made from plastic mesh. The other was a metal-baffle arrangement which satisfactorily prevented the escape of the moths, but caused some undesirable back pressure on the fan.

A large percentage of the energy radiated by the black-light lamps was between 3200 and 4000 Angstroms with a peak radiation around 3650 A. These lamps were similar in appearance to other preheat fluorescent lamps of equivalent wattages and operated on the same auxiliary equipment. The 15-watt lamp was 18 inches long, and the 30-watt unit was 36 inches long. Both lamps were 1 inch in diameter. The 15-watt lamp had an approximate radiant energy output of 1950 milliwatts, or fluorens, and the 30-watt lamp had an output of 5100 fluorens, as reported by Weitz (1956).

EXPERIMENTAL PROCEDURE.—At each of 3 locations, 3 traps were placed in a tobacco field of approximately 5 acres in size at strategic points with respect to visibility and distance from lamps. All traps in a particular field were of the same type throughout the 3 years the investigations were in progress. The suction-type traps were used at location A, and the mechanical type at B and C, as referred to in tables 1 and 2.

The traps were placed in operation during the time the tobacco was being transplanted to the field and were operated continuously until it had been harvested. Traps were visited twice weekly, except during the peak of the moth flight, at which time three visits were necessary. Captured hornworm moths were identified as to species and sex at the time they were removed from the collect-

Table 1.—Tobacco- and tomato-hornworm moths caught in black-light traps at three locations near Chatham, Va., 1954–56, inclusive.

Location[a]	*P. sexta*		*P. quinquemaculata*		Per Cent of Moths *P. sexta*
	Number	Per Cent Female	Number	Per Cent Female	
		1954			
A	971	22.5	1,047	37.6	48.1
B	2,212	19.7	1,770	44.8	55.5
C	1,690	16.7	1,963	42.8	46.3
Total	4,873	19.2	4,780	42.4	50.5
		1955			
A	957	16.6	2,155	45.9	30.7
B	5,015	20.1	5,311	46.7	48.6
C	3,871	14.5	4,262	48.4	47.6
Total	9,843	17.6	11,728	47.2	45.7
		1956			
A	1,402	15.4	3,945	47.1	26.2
B	2,024	18.2	7,351	48.4	21.6
C	1,945	16.2	7,335	50.0	20.9
Total	5,371	16.8	18,631	48.7	22.4

[a] Three traps at each location.

Table 2.—Tobacco plants damaged by hornworms in three tobacco fields in which black-light traps were used and in check fields.

Location	Date Plants Examined	Per Cent of Plants Damaged in Test Fields	Per Cent of Plants Damaged in Check Fields	Plant Damage Ratio
	1954			
A	June 24	1.4	5.3	0.264
	Aug. 24	1.0	3.0	0.333
B	June 24	1.5	4.6	0.326
	Aug. 24	4.7	3.6	1.306
C	June 24	4.3	3.9	1.103
	Aug. 24	2.8	3.1	0.903
	1955			
A	June 22	8.6	11.6	0.741
	Aug. 16	12.3	14.2	0.866
B	June 22	8.1	8.2	0.988
	Aug. 16	14.1	20.5	0.688
C	June 22	7.7	10.0	0.770
	Aug. 16	9.3	12.1	0.769
	1956			
A	July 3	10.4	19.3	0.539
B	July 3	16.3	16.8	0.970
C	July 3	28.3	35.0	0.809
	Sept. 5	3.8	5.0	0.760

ing bag. The suction-type traps damaged many moths beyond identity; however, it is estimated that at least two-thirds of each catch were identified.

Field examinations were made from time to time to note hornworm activity. Two plant-injury counts were made, the first in late June or early July following the peak of the first flight and the second in late August or early September following the peak of the second flight. These counts were made in 1954 by examining 100 consecutive plants in a row at random locations with respect to the distance from the trap; but in 1955 this method was changed, and 20 consecutive plants were examined in each of 5 rows in a block at random locations. All plants which showed one or more leaves injured as a result of hornworm feeding were scored as damaged. Two or three check fields in the same vicinity and in approximately the same stage of growth were checked for comparison.

MOTH COLLECTIONS.—A summary of tobacco- and tomato-hornworm moth collections and the proportion of sexes is presented in table 1. It may be noted that the proportion of species among the moths caught in the nine traps during 1954 was similar: approximately 50% were the tobacco hornworm. In 1955 there was an increase of about 50% in the number of tobacco-hornworm moths captured and an increase of 63% in the number of tomato-hornworm moths. These data show a gradual decrease in the number of female tobacco-hornworm moths captured: the 3-year average being only 17.9%. This low percentage of females is in general agreement with Stahl (1954), who reported an average of only 21% for five seasons. Madden & Chamberlin (1954), in studies on the biology of the tobacco hornworm, concluded that the sexes are approximately equal in numbers under natural conditions. In a study of the mating habits of the tobacco hornworm, Allen & Hodge (1955) concluded that, since the male will fertilize more than one female, it is unlikely that females will remain unfertilized under natural conditions.

It is of interest to note that, with the corresponding increase in the number of tomato-hornworm moths, there was also an increase in the number of females of this species caught. During the 3 years while the work was in progress, 45.8% of the tomato-hornworm moths caught were female.

RESULTS OF DAMAGE.—The results of the tobacco plant damage counts are given in table 2. In order to further evaluate these data, a ratio of the test field to the check field has been determined and is shown in the table.

It will be noted that a heavy infestation did not occur during any of the 3 years of these studies. The infestation of the two broods during 1954 was unusually light, but each was somewhat heavier in 1955 and 1956. The heaviest infestation occurred in 1956 at location C, where 28.3% of the plants were damaged by hornworms while 35% of the plants were damaged in the check fields. During the period of May 18 to June 30, a total of 88 female tobacco-hornworm and 1917 female tomato-hornworm moths were taken at location C in the three traps.

In two instances the percentage of damaged plants was greater in the test field than in the check fields, and in several other cases it was only slightly reduced. In 1955 the first field counts showed a gradual increase in the severity of the infestation as the distances from the traps increased; but the second field count for that year and both counts in 1956 failed to substantiate these data.

REFERENCES CITED

Allen, Norman, and C. R. Hodge. 1955. Mating habits of the tobacco hornworm. Jour. Econ. Ent. **48**(5): 526–9.

Dominick, C. B. 1950. Hornworm control on tobacco. Jour. Econ. Ent. **43**(2): 221–2.

Dominick, C. B. 1951. Tests with insecticides for hornworm control on tobacco. Jour. Econ. Ent. **44**(4): 539–41.

Glick. P. A., and J. P. Hollingsworth. 1956. Further studies on the attraction of pink-bollworm moths to ultraviolet and visible radiation. Jour. Econ. Ent. **49**(2): 158–61.

Haeussler, G. J. 1952. Insects as destroyers: Losses caused by insects. *In* U. S. Dept. Agric. Yearbk. **1952**, pp. 141–6.

Madden, A. H., and F. S. Chamberlin. 1945. Biology of the tobacco hornworm in the southern cigar-tobacco district. U. S. Dept. Agric. Tech. Bull. **896.**

Pfrimmer, T. R., M. M. Lukefahr, and J. P. Hollingsworth. 1955. Experiments with light traps for control of the pink bollworm. U. S. Dept. Agric., Agric. Res. Serv. **33–6.**

Stahl, C. R. 1954. Trapping hornworm moths. Jour. Econ. Ent. **47**(5): 879–82.

Taylor, J. G., and H. O. Deay. 1950. Electric lamps and traps in corn borer control. Agric. Engineer. **31**: 503–5.

Weitz, C. E. 1956. Lamp Bull., LD-1, second printing, General Electric Co.

Transient Insects in Delaware's Apple and Peach Plantings[1]

L. A. Stearns, *University of Delaware, Newark*

The insect complex associated with a particular crop presents an amazing array of component species at different times throughout the year. For example, in orchard districts of the Middle Atlantic States, the codling moth (*Carpocapsa pomonella* (L.)), oriental fruit moth (*Grapholitha molesta* (Busck)), plum curculio (*Conotrachelus nenuphar* (Hbst.)) and red-banded leaf roller (*Argyrotaenia velutinana* (Wlk.)) are recognized as ever-present major pests of apple and/or peach, and the spray programs for these fruits are reconstructed annually with effective control of this group the principal objective.

Of secondary importance, but frequent threats to successful production, are scale insects (Forbes, San Jose, scurfy and terrapin), aphids (grain, green and rosy), plant bugs (several kinds), mites (European red and two-spotted), unspotted tentiform leaf miner (*Callisto geminatella* (Pack.)), Japanese beetle (*Popillia japonica* Newm.) and certain others. The occurrence of all of these from year to year and seasonal abundance as well are affected by many factors, including the activity of natural enemies, weather and managerial practices. Therefore, materials for their control are incorporated regularly in the fruit-spray programs and the scheduling of applications is such as to provide for possible contingencies.

This paper is concerned with the even-less-well-known, continually-changing, migrant segment of an orchard's population. These insects are present today; may be absent tomorrow; and many of them, obviously, have host affinities elsewhere. However, when encountered either singly or in numbers, the following questions logically arise—is this species here by choice or by chance; and does its appearance in this planting augur well or ill for the grower concerned?

An instance of this kind was the subject of an earlier article (Stearns 1956), in which evidence was submitted definitely incriminating the meadow spittlebug (*Philaenus leucophthalmus* (L.)) as the agent responsible for a distinctive type of peach gumosis. In this 1955 study of what had long been an elusive problem, sticky-board traps (fully described in that report) served as a device for tracing the movement of spittlebug adults into the peach blocks under observation. Many other interesting insect captures were made and commented upon.

Since some of these were well-known pests of other locally-important crops and additional information of this nature seemed desirable, these same traps were used again in 1956. On April 10, 30 of them were suspended in plantings at each of three locations, as follows: Camden, Lord orchard, 17-year-old Elberta peach, pink to partial-bloom stage; Bridgeville, Smith orchard, 19-year-old Williams apple, early green-tip stage; and Georgetown, University Substation orchard, 11-year-old Lodi, early pre-pink stage. The traps were examined periodically (as many as 10 times) until their removal on July 24 to 26 and, on each occasion, the insects present were either recorded or extracted from the adhesive for determination later. The 35 species taken are listed in table 1. It may be noted that 28 of these were trapped on peach, compared with only 15 on apple; also, that occurrences totaled 72, and the number collected, 186.

Table 1.—Insects captured by sticky-board traps in apple (Bridgeville and Georgetown) and peach (Camden) plantings, Delaware, 1956.

Species[a]	Collected		Trapped on	
	Times	Number	Apple	Peach
Sehirus cinctus (P. de B.)	1	1		x
Euchistus tristigmus (Say)	1	1	x	
Lygus lineolaris (P. de B.)	2	2		x
Deraecoris historio (Reut.)	2	2		x
Monecphora bicincta (Say)	1	1	x	
Philaenus leucophthalmus (L.)	5	50	x	x
Oncometopia undata (F.)	7	25	x	x
Graphocephala versuta (Say)	5	13	x	x
Draeculacephala mollipes (Say)	3	3		x
Gypona octolineata (Say)	1	1	x	
Norvellina seminuda (Say)	2	2		x
Paraphlepsius irroratus (Say)	2	8		x
Colladonus clitellarius (Say)	1	1		x
Agonoderus comma (F.)	1	1		x
Coleomegilla fuscilabris Muls.	3	3		x
Cycloneda munda (Say)	4	5	x	x
Adalia bipunctata (L.)	2	2		x
Chilocoris bivulnerus Muls.	1	1		x
Stenotarsus hispidus (Hbst.)	1	1		x
Ptilodactyla serricollis (Say)	1	1		x
Monocrepidius vespertinus (F.)	1	1	x	
Aphodius distinctus (Müll.)	1	1		x
Neoclytus acuminatus (F.)	1	1		x
Crioceris asparagi (L.)	1	2	x	
Crioceris duodecimpunctata (L.)	2	3	x	x
Arthrochlamps plicata (F.)	1	1		x
Cerotoma trifurcata (Forst.)	1	1		x
Galerucella notulata (F.)	1	2		x
Diabrotica undecimpunctata howardi Barb.	3	3	x	x
Acalymma vittata (F.)	1	1	x	
Disonycha pennsylvania (Illig.)	2	4		x
Metriona bicolor (F.)	5	23	x	x
Conotrachelus nenuphar (Hbst.)	1	1		x
Chaetopsis fulvifrons (Macq.)	1	1	x	
Syrphus americanus Wied.	4	17	x	x
Totals—35	72	186	15	28

[a] Determinations either made or confirmed by Dr. H. E. Milliron.

The table contains four species of Heteroptera (Cydnidae, Pentatomidae and Miridae), nine species of Homoptera (Ceropidae and Cicadellidae), 20 species of Coleoptera (Carabidae, Coccinellidae, Endomychidae, Dascyllidae, Elateridae, Scarabaeidae, Cerambycidae, Chrysomelidae and Curculionidae) and two species of Diptera (Otitidae and Syrphidae). The Chrysomelidae and Cicadellidae are represented best, with nine and seven species, respectively. Notably absent, are members of the orders, Hymenoptera and Lepidoptera. This listing does not

[1] Published as Miscellaneous Paper No. 287, with the approval of the Director of the Delaware Agricultural Experiment Station. Publication 285 and Scientific Article 203 of the Department of Entomology, August 16, 1957. Accepted for publication August 23, 1957.

include these observations: April 17—a few adults of the unspotted tentiform leaf miner at Georgetown; April 23—many, unremovable, minute Diptera, but only Syrphids recognizable at Camden; and June 19—lacewing-flies (Neuroptera, Chrysopidae) abundant at all three points.

Of the major pests of apple and peach, a single specimen of the plum curculio was recorded from peach at Camden on May 2; this was the date of peak abundance of overwintered adults as determined by jarring. Of the insects usually of secondary importance, only unspotted tentiform leaf miner moths were recorded from apple at Georgetown on April 17.

Of the transient population, the meadow spittlebug was most numerous, but less so than in 1955. It was taken from both apple and peach between June 19 and July 26, compared with May 17–23 to July 25–31 in 1955. The earliest mature forms of this species were encounted (by Dr. H. E. Milliron) in alfalfa and clover fields on May 16 in 1955, but not until June 4 in 1956; however, the characteristic, spittlebug-produced, string-like, gummy exudations from peach fruit appeared first at the same time both years (during the third week in June). Single specimens only of the tarnished plant bug (*Lygus lineolaris* (P. de B.)) and of the dusky stink bug (*Euchistus tristigmus* (Say)) were taken. These species are known to be responsible for peach "cat-facing" (Rice 1937, Chandler 1955). Since *Sehirus cinctus* (P. de B.) has been recorded from the flowers of wild plum and from wild cherry (Blatchley 1926), its presence on peach is understandable.

The four Coccinellids and *Syrphus americanus* Wied. are, of course, beneficial insects and were engaged actively in their quest for aphids. The former, according to Blatchley (1910), are "predaceous in both the larval and adult stages and seem to be especially adapted to keeping in check the extensive families of plant lice," while Metcalf (1916) ranks the latter as "one of our most valuable predaceous forms."

Records for occasional asparagus beetles, spotted asparagus beetles, bean leaf beetles, spotted cucumber beetles, striped cucumber beetles and golden tortoise beetles are not surprising in view of substantial plantings in their host crops (asparagus, 2650 acres; all beans, 176,600; all cucurbits, 4600; and sweet potatoes, 1000). In each case, captures were made during the period of maximum abundance and activity on such hosts (between June 8 and 29).

The remaining nine species of Coleoptera and the seven Cicadellids (all described as general feeders on weeds, grasses and hay crops) apparently have no tangible association with either apple or peach.

These notes extend somewhat present meager knowledge of the migrant insect population in Delaware's orchards.

REFERENCES CITED

Blatchley, W. S. 1910. Coleoptera or beetles known to occur in Indiana. The Nature Pub. Co., Indianapolis: 506–33.

Blatchley, W. S. 1926. Heteroptera or true bugs of eastern North America. The Nature Pub. Co., Indianapolis, 90–1.

Chandler, S. C. 1955. Biological studies of peach cat-facing insects in Illinois. Jour. Econ. Ent. **48**(4): 473–5.

Metcalf, C. L. 1916. Syrphidae of Maine. Maine Agric. Expt. Sta. Bull. **253**: 236–40.

Rice, Paul L. 1937. Cat-facing of peaches by the tarnished plant bug, *Lygus pratensis* (L.) Trans. Peninsula Hort. Soc. Bull. Delaware State Board Agric. **27**(5): 131–6.

Stearns, L. A. 1956. Meadow spittlebug and peach gumosis. Jour. Econ. Ent. **49**(3): 382–5.

Orchard Studies on the Effect of Organic Insecticides on Parasitism of the Oriental Fruit Moth[1]

H. W. ALLEN, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*[2]

ABSTRACT

Studies on the effect of organic insecticides on parasitism of the oriental fruit moth (*Grapholitha molesta* (Busck)) were made from 1948 through 1953 in peach orchards near Moorestown, N. J. In orchards not receiving such insecticides parasitism of twig-infesting larvae of the first two generations was as high as it was before they came into general use. It was highest in the orchard groups where there was no exposure and lowest in those having a heavy exposure to these insecticides. Significant reductions in parasite levels did not always follow heavy exposure to these insecticides, and after spray applications were discontinued for the season, both moth and parasite populations usually regained normal levels within a few weeks. The average reduction in parasitism, even in orchards heavily exposed, was usually less than 50%.

Only the sprays applied before July 10 directly affected parasitism of the twig-infesting larvae. These included the early sprays used on peach, such as BHC, DDT, and the phosphorus insecticides used for control of the plum curculio or the oriental fruit moth, and similar sprays used on adjoining crops and orchards. There were instances in individual orchards of greatly reduced parasitism following moderate to heavy exposure to each of them.

Several workers have shown by laboratory and cage tests that some of the common parasites of the oriental fruit moth (*Grapholitha molesta* (Busck)) are susceptible to small deposits of organic insecticides such as DDT and parathion and are more readily killed by them than is the oriental fruit moth. However, the actual effect in peach orchards can be obtained only by studies of the orchard

[1] Accepted for publication August 29, 1957.

[2] E. L. Plasket assisted in the work on which this paper is based. Some of the observations are based on data on fruit moth injury obtained by M. H. Brunson.

populations. This is a report on such studies made in the vicinity of Moorestown, N. J., from 1948 through 1953.

Only a few other studies of a similar nature have been reported. Wheeler & LaPlante (1946), in observations within one large orchard, found that there was a large reduction in parasitism of the first generation in plots treated with DDT, and predicted that applications every 2 to 3 weeks would practically exterminate the parasites attacking the oriental fruit moth in New York State. After 3 weeks without spraying there was a rapid rise from the first to the second generation in both moth and parasite populations, which they suspected was due to infiltration from surrounding areas. Rings & Weaver (1948), in 40 peach orchards in several counties in Ohio, observed that BHC used in early sprays to control the plum curculio did not reduce the activity of the parasites of the oriental fruit moth, that parasitism was much lower in orchards where DDT was used, and that the use of DDT one year might cause a reduction in parasitism the following year. Boyce & Dustan (1955) noted that in Ontario *Macrocentrus ancylivorus* Roh. and *M. delicatus* Cress. had increased and *Glypta rufiscutellaris* Cress. and *Horogenes obliteratus* (Cress.) decreased since DDT and parathion came into general use there.

At Moorestown the effect of several chlorinated hydrocarbon and phosphorus insecticides on the parasites of the oriental fruit moth has been studied by comparing rates of parasitism in peach orchards before and after these insecticides came into general use. The parasites that attack oriental fruit moths in peach orchards are part of much larger populations of the same parasites which also attack several other lepidopterous insects infesting wild and cultivated strawberry and blackberry and some of the common weeds. None of these plants ordinarily receive deposits of the organic insecticides. If these insecticides were reducing significantly the general level of parasite population, parasitism in orchards that are not now being sprayed with them should be less than it was before these insecticides came into general use. Studies were also made in orchards where, within or adjacent to them, the insecticides were or were not used. The relations of population levels of the oriental fruit moth and its parasites in peach to their occurrence in other hosts, and of population densities of oriental fruit moths to rates of parasitism, are discussed.

PARASITISM BEFORE AND AFTER ORGANIC INSECTICIDES CAME INTO GENERAL USE.—Observations on parasitism in twig-infesting larvae have been made for many years in Burlington County, New Jersey. Some of these observations were made in recent years in orchards not receiving the new organic insecticides, at least not until after the twig samples had been taken. Thus they provide the best available basis for comparing over-all parasitism now existing with that occurring before the new insecticides became generally used. Several collections of infested twigs were made in each orchard during each generation, and the adult moths and parasites were allowed to emerge. The per cent parasitism was obtained for each collection, and the percentages were averaged for each generation. Table 1 shows that the parasitism in orchards studied from 1948 to 1953 was approximately the same as that in those studied from 1939 to 1942. There is thus no evidence that the use of organic insecticides has reduced the over-all parasite populations.

Table 1.—Parasitism of oriental fruit moth larvae in orchards before and after organic insecticides came into general use.

Year	First Generation		Second Generation	
	Number of Orchards	Per Cent of Larvae Parasitized	Number of Orchards	Per Cent of Larvae Parasitized
Before insecticides were used				
1939	9	49	9	67
1940	4	27	4	66
1941	4	36	4	73
1942	6	78	6	74
After insecticides were used				
1948	3	54	3	84
1949	6	56	3	67
1950	4	50	3	77
1951	—	—	9	77
1953	3	64	3	72

PARASITISM IN RELATION TO EXPOSURE TO ORGANIC INSECTICIDES.—Our survey involved the effect of sprays applied from about May 1 to July 10. During this period the first and second generations of fruit moth larvae occur, mostly in the twigs, and the most important parasitism occurs during this part of the season. Although parasites of the eggs and cocoons may at times be important, no information was obtained on such species.

At the time of these studies BHC, DDT, and phosphorus insecticides, such as parathion, EPN, and Metacide, were being used in peach orchards. The spray schedule varied from orchard to orchard and from year to year. At first BHC was employed at the shuck-split stage and for 20 days thereafter for plum curculio control, but recently there has been a strong trend toward the use of phosphorus sprays in May and early June. DDT has been applied in both early and preharvest sprays, but recently mainly in a preharvest spray.

The orchards under observation were classified in three groups—(1) those treated with organic insecticides just before or during the period of observation, (2) those not treated but located within 200 yards of an orchard or other crop that was dusted or sprayed during the observation period, and (3) those in which none of these sprays were used either in the orchard or within 200 yards of it. The second group included a few that had been sprayed for control of the first generation, but treatment had been discontinued several weeks before second-generation twig collections were started. The three groups have been designated, respectively, as heavily exposed, moderately exposed, and not exposed.

Since parasites fly freely, and are presumed to be continually drifting in and out of any particular orchard, the use of insecticides in the adjacent fields and orchards might reduce parasite populations within the orchard being studied. Various kinds of crops were being grown within the 200-yard belt about the experimental orchards. They included other fruits (peach, apple, or cherry), truck crops, grains, forage crops, and pasture. The grains, forage, and pasture were not treated with insecticides. Summer squash was treated with insignificant quantities when the plants were very small. Several, as tomato, were treated only after our observations. Except where applied

Table 2.—Parasitism of the oriental fruit moth in orchards in Burlington County, N. J., having different degrees of exposure to organic insecticides.

Year and Generation	No Exposure			Moderate Exposure			Heavy Exposure		
	Orchard No.	Number of Moths and Parasites Emerged	Per Cent Parasitism	Orchard No.	Number of Moths and Parasites Emerged	Per Cent Parasitism	Orchard No.	Number of Moths and Parasites Emerged	Per Cent Parasitism
1948									
First	1	343	64	—	—	—	4	367	19[a]
	2	433	43	—	—	—	5	130	23[a]
	3	400	55	—	—	—	6	370	18[a]
Second	1	305	81	5	218	78[b]	—	—	—
	2	314	84	6	329	70[b]	—	—	—
	3	231	86	—	—	—	—	—	—
1949									
First	3	194	63	11	129	55	12	266	69[a]
	6	189	49	—	—	—	13	29	16[a]
	7	224	42	—	—	—	—	—	—
	8	194	55	—	—	—	—	—	—
	9	186	63	—	—	—	—	—	—
	10	225	63	—	—	—	—	—	—
Second	3	203	61	7	107	41	4	45	56[c]
	6	278	57	9	206	78[d]	12	251	62[c]
	8	192	84	10	204	76	13	190	42[c]
	—	—	—	11	180	64	—	—	—
	—	—	—	14	172	38	—	—	—
1950									
First	3	169	67	15	67	50	10	19	14[a]
	8	91	29	16	104	73	11	57	44[a]
	9	44	46	—	—	—	13	9	0[a]
	12	57	58	—	—	—	14	11	69[a]
Second	3	129	74	6	56	27	10	140	58[a]
	8	150	78	7	50	38	12	59	76[a]
	9	45	78	11	235	86[b]	—	—	—
	—	—	—	13	145	59[b]	—	—	—
	—	—	—	14	55	58	—	—	—
	—	—	—	15	93	72	—	—	—
	—	—	—	16	228	78	—	—	—
1952									
First	17	65	89	16	12	58	12	6	50[e]
	—	—	—	18	33	79	20	41	90[e]
	—	—	—	19	1	0	21	36	83[a]
	—	—	—	—	—	—	22	5	81[e]
	—	—	—	—	—	—	23	15	48[e]
Second	17	31	90	12	2	50	19	4	0[e]
	—	—	—	16	11	83	—	—	—
	—	—	—	20	66	41[b]	—	—	—
	—	—	—	21	28	72[b]	—	—	—
	—	—	—	22	24	46	—	—	—
	—	—	—	23	7	58	—	—	—
	—	—	—	27	18	34	—	—	—
Average for 5 years:									
First generation			56.1			52.5			44.6
Second generation			77.3			60.6			49.0

[a] Sprayed with BHC.
[b] Probable residues from earlier spraying.
[c] Sprayed with parathion or other phosphorus insecticide.
[d] Trunks only sprayed with DDT.
[e] Sprayed with DDT.

in small amounts or after second-generation oriental fruit moth had completed its development, insecticide applications in any of the crops in the adjoining belt could have affected the parasite population in the experimental orchards, especially DDT dust on sweet corn and chlorinated hydrocarbon or phosphorus insecticides on peach or apple.

In each orchard two or more samples of infested twigs were taken at the peak of each generation except in 1952 when only one collection was made during the second

generation. Whenever feasible, the sample consisted of 200 twigs, but in orchards where twigs were scarce the samples were smaller.

The results of this survey are presented in table 2. In 1952 the twig infestation was exceptionally low, and the time spent in making collections had to be reduced; hence the numbers of moths and parasites that emerged from some twig collections were so small that the rates of parasitism obtained are not highly reliable. In the other years the number of insects reared exceeded 30 in all but four of the observations. The total number in all the observations included in this table exceeded 9,500.

In both generations the average parasitism was less in orchards with a moderate exposure than in those with no exposure, and less in orchards with a heavy exposure than in those with a moderate exposure. The differences were significant at the 1% level in the second generation but not in the first. In orchards with a heavy exposure the average parasitism was more than half that in the orchards having no exposure. The highest rate in the non-exposed group—90% in orchard 17, second generation, 1952—was not significantly higher than the 86% in the group having a moderate exposure (orchard 11, second generation, 1950), and no higher than that in the group having heavy exposure (orchard 20, first generation, 1952). However, there were seven heavily exposed orchards having less than 30% parasitism, two in the group with moderate exposures, and only one in those not exposed. In some of the sprayed orchards there was a considerable reduction in parasitism, but in others the parasitism remained high.

It is well known that phosphorus residues are soon dissipated but that those of the chlorinated hydrocarbons may persist for several weeks. Several orchards that received BHC or DDT sprays early in the season (orchards 5 and 6 in 1948, 11 and 13 in 1950, and 20 and 21 in 1952), and had heavy exposure during the first generation but were not sprayed against later generations, were placed in the group with moderate exposure during the second generation, because of the probability that they still had residues persisting from early spraying. In all these orchards parasitism of the second generation was close to average levels. This indicates a tendency to recover quickly from depressed parasitism following early-season spraying. In the single orchard in which DDT was sprayed in midsummer as a trunk wash for borer control (orchard 9, 1949), there was no reduction in parasitism.

The average first-generation parasitism in the nine orchards receiving early sprays of BHC (orchards 4–6, 10–13, 20, and 21) was 41.8%, considerably less than the 56.1% in the orchards receiving no early sprays of organic insecticides. However, three of these orchards showed higher parasitism than the average in the non-exposed orchards. Thus, the evidence is that the BHC sprays did reduce the average rate of parasitism. The data on some of these orchards differ from those of Rings & Weaver (1948) for orchards in Ohio in showing much lower than average parasitism. This may be due to the fact that our first-generation collections were made during or shortly after the spraying period, whereas their collections apparently continued long after the final application of BHC.

Stronger evidence of the effect of spraying with BHC was obtained in 1948 than in the subsequent years. The rates of parasitism in three orchards receiving early applications of BHC were compared with those in three orchards receiving arsenical sprays but no organic insecticide. Since fairly large numbers of insects emerged, the estimate of parasitism was restricted to the larvae maturing within 5 days after the sample was collected. Nearly mature larvae provide the best estimate of parasitism, since they have been exposed for nearly the longest possible period. About 95% of the parasites reared were *Macrocentrus ancylivorus*. Three collections were made during the first generation and two during the second.

Table 3 shows that in each collection period the parasitism in the orchards sprayed with BHC was less than in the check orchards. The differences were significant only in the first and second collections. By the time the first of the second-generation collection was made about 5 weeks had elapsed since the final BHC spray, and

Table 3.—Effect of BHC sprays on parasitism of oriental fruit moth larvae in orchards in 1948.

Orchard No.	Dates Sprayed	First Generation Larvae Collected on: May 24–27 — Number of Moths and Parasites Emerged	May 24–27 — Per Cent parasitism	June 1–4 — Number of Moths and Parasites Emerged	June 1–4 — Per Cent Parasitism	June 7–9 — Number of Moths and Parasites Emerged	June 7–9 — Per Cent Parasitism	Second Generation Larvae Collected on: June 28–July 1 — Number of Moths and Parasites Emerged	June 28–July 1 — Per Cent Parasitism	July 6–7 — Number of Moths and Parasites Emerged	July 6–7 — Per Cent Parasitism
Sprayed with BHC											
4	May 27, 29	58	52	64	23	63	6	—	—	—	—
5	May 7, 15, 24	4	50	17	24	36	17	53	89	13	92
6	do.	33	24	51	24	53	26	81	52	73	86
Average			42.0		23.7		16.3		70.5		89.0
Checks (sprayed with arsenical)											
1	—	33	79	28	86	69	90	—	—	—	—
2	—	37	78	55	65	65	22	118	96	35	97
3	—	29	76	91	69	83	54	29	90	108	98
Average			77.7		73.3		55.3		93.0		97.5

Table 4.—Effect of DDT sprays on parasitism of oriental fruit moths in peach twigs from plots sprayed with DDT in 1948.

Date		Sprayed		Unsprayed	
Sprayed	Collected	Number of Moths and Parasites Emerged	Per Cent Parasitism	Number of Moths and Parasites Emerged	Per Cent Parasitism
May 18	May 25	1	100	12	50
	28	0	—	13	62
28	June 3	1	100	45	73
	7	1	0	29	79
	11	11	64	43	67
	18	24	67	32	69
June 21	June 23	79	33	37	46
	28	36	72	63	70
	July 2	18	67	34	71
July 2	7	34	88	60	85
	14	21	86	49	94
	19	21	33	31	55
	22	19	74	7	29
Total or average		266	59.4	455	70.8

parasitism in the sprayed orchards had returned to high levels.

STUDY OF INDIVIDUAL ORCHARDS.—In 1948 observations were made on the effect of DDT on parasitism in a large 4-year-old orchard. A plot of 29 trees was sprayed twice against each of the first two generations, and a check plot of 14 unsprayed trees was separated from it by four buffer rows. Both plots had similar positions in respect to outside and inside trees.

The results are shown in Table 4. In the sprayed plot the twig infestation was very light until June 11, but moderate thereafter. Parasitism was fairly high and not significantly less than in the unsprayed plot. It seems probable that the toxic effect of the DDT, which was used in a relatively small part of the orchard, was not great enough to upset the tendency of migrating parasites to maintain a balance in all parts of the orchard.

About 95% of the parasites reared were *Macrocentrus ancylivorus*, and most of the rest were *M. delicatus*. The per cent of *delicatus* reared from the sprayed plot was 2.5, compared with 6.5 from the unsprayed plot. This suggests that *delicatus* was more susceptible to the DDT than *ancylivorus*.

In 1949 and 1950 the degree of exposure to organic insecticides was studied in 23 orchards. Data from three of these orchards, taken as typical examples, are presented below.

In orchard 13, studied in 1949, the test block was trapezoidal and contained about 550 bearing trees (fig. 1). It received in May BHC for curculio, and about June 10 DDT and TEPP. Adjoining it on the north, northeast, and east was a broad exposure to peach trees having the same treatment as the test block. The apples to the east and southeast received a DDT spray in May. In addition there was sweet corn on all sides of the test block, which received about five applications of DDT dust, in April, May, and possibly in June. The parasites in both the test block and its adjoining belt were heavily exposed to organic insecticides in both generations.

Three collections of infested twigs were made during the first generation, from which the following results were obtained: May 19, 9 insects reared and none parasitized; May 27, 9 reared and 22% parasitized; June 2, 11 reared and 27% parasitized. Two collections during the second generation gave the following results: June 29, 109 reared and 68% parasitized; July 7, 81 reared and 16% parasitized.

When infestation was sparse, at least 2 man-hours were spent in each orchard in an attempt to obtain 100 twig-infesting larvae. Only a few first-generation moth larvae were found, and in these parasitism was relatively low. However, by June 29, about 19 days after the final application in the test orchard, both moth infestation and parasitism had increased to normal levels.

Orchards 6 and 15 were studied in 1950. Orchard 6 received parathion sprays on May 12 and 23 and June 2, and thereafter no insecticide. Orchard 15, lying partly within the 200-yard belt about orchard 6, contained young trees that received no insecticide. Both orchards were adjoined by apple receiving three early parathion sprays and two cover sprays of DDT in June and early July. Thus, both orchards had moderate exposure to insecticides in the adjoining belt, and orchard 6 heavy exposure during the first generation. In moderate to heavy infestations at least 100 insects are generally collected per hour.

The results are shown in table 5. Despite its proximity to orchards heavily sprayed with parathion, the unsprayed orchard showed a fair population of oriental fruit moths and a moderate rate of parasitism in both generations. In the sprayed orchard the oriental fruit moth was almost completely eliminated during the first generation, but began to re-establish itself in the second. As soon as the population had risen to a moderate level, parasitism jumped to a rate only slightly lower than that in the unsprayed orchard. It is probable that a large portion of the second-generation moths and parasites in the sprayed orchard were the progeny of migrants from nearby unsprayed areas.

DISCUSSION AND CONCLUSION.—When phosphorus insecticides are used against the early generations, the moth and associated parasite populations are nearly eliminated

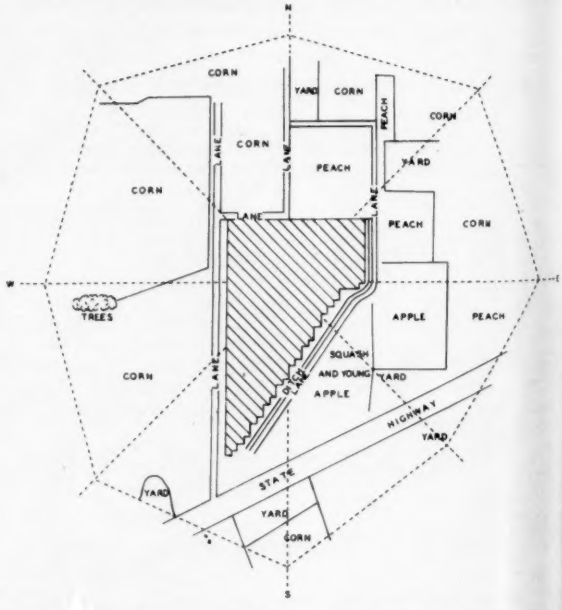


FIG. 1.– Map of orchard 13 and its adjoining belt in 1949. The hatched area is the experimental block.

from peach trees for a few weeks. The great reduction in moth population and parasites presumably makes it more difficult for the few remaining parasites to find the few remaining infested twigs. In this respect insecticides seem to have a considerable indirect effect in reducing parasitism. Low parasitism in sparse populations is frequently observed. For instance, in 1952 estimates of the moth populations were obtained when all the infested twigs were collected on a certain number of sample trees. In one group of five orchards having a heavy to moderate exposure to organic insecticides, there was an average population of 18 oriental fruit moth larvae per acre (only one to five trees) and a parasitism of 47%. In another group of four orchards having about the same exposure, the average moth population was 162 per acre and the parasitism 85%. It seems reasonable to suppose that in the first group parasites would be unable to find as large a portion of the larvae as in the second group.

Since the populations of both the oriental fruit moth and its parasites in peach orchards normally fall to a low level after the crop is harvested in August or early September, the orchards must be restocked more or less continuously from other breeding areas within migrating distance. Neglected unsprayed apple trees produce large numbers of oriental fruit moths. The ragweed borer (*Epiblema strenuana* (Wlk.)) (Allen & Lott 1930) and the strawberry leaf roller (*Ancylis comptana fragariae* (W. & R.)) (Allen 1945) produce large numbers of oriental fruit moth parasites on vegetation not usually contaminated by appreciable amounts of insecticide. This situation may explain why both moth and parasite populations in orchards where the new insecticides are not used have remained relatively high despite their widespread use in adjoining orchards and fields of truck crops. It may also help to explain the abrupt rise of moth populations and parasitism in orchards shortly after applications have been discontinued.

Table 5.—Infestation and parasitism of the oriental fruit moth in two adjoining experimental orchards, 1950.

Date Twigs Collected	Sprayed with Parathion			Unsprayed Orchard		
	Number of Larvae Collected per Hour	Number of Moths and Parasites Emerged	Per Cent Parasitism	Number of Larvae Collected per Hour	Number of Moths and Parasites Emerged	Per Cent Parasitism
		First generation				
June 5	0	0	—	142	45	78
13	0	0	—	55	22	23
		Second generation				
July 3	5	5	0	8	4	75
11, 12	46	51	55	97	89	68

Our observations indicate that the potential capacity of parasites to reduce oriental fruit moth populations has not been appreciably affected by the general use of the new insecticides, and that large numbers of moths will continue to be destroyed by them in orchards not sprayed, and also in those that are sprayed, as soon as their residues have weathered to a point permitting the reestablishment of the fruit moth infestation.

References Cited

Allen, H. W. 1945. Sources of overwintering *Macrocentrus ancylivorus*. Jour. Econ. Ent. **38:** 119–20.

Allen, H. W., and Earl Lott. 1930. *Epiblema strenuana* Wlk., host of certain parasites of the oriental fruit moth, *Laspeyresia molesta* Busck. Proc. Ent. Soc. Wash. **32:** 135–6.

Boyce, H. R., and G. G. Dustan. 1955. Parasitism of twig-infesting larvae of the oriental fruit moth *Grapholitha molesta* (Busck) in Ontario. Ent. Soc. Ont. Ann. Rept. **84:** 48–55.

Rings, R. W., and C. R. Weaver. 1948. Effects of benzene hexachloride and DDT on parasitization of the oriental fruit moth. Jour. Econ. Ent. **41:** 566–9.

Wheeler, E. H., and A. A. LaPlante. 1946. DDT and Ryanex to control oriental fruit moth. Their effect on parasite populations. Jour. Econ. Ent. **39:** 211–5.

Holly Bud Moth Control[1]

K. G. Swenson, *Oregon State College, Corvallis*

Abstract

The holly bud moth, *Rhopobota naevana* (Hbn.) Kearfott, was introduced into British Columbia before 1923 and has subsequently spread southward through Washington into Oregon. It is now an important pest in Oregon holly plantings. Evaluation of insecticides for holly bud moth control showed that a high degree of control can be obtained with organic phosphate insecticides. Malathion consistently provided excellent control with one spray applied sometime between the opening of the leaf buds and the time of blossoming. Good control was also obtained with methoxychlor. DDT did not give consistent results under the same conditions. The difference between the results with the organic phosphates and with DDT might be explained on the basis of DDT tolerance in the holly bud moth. Another possible explanation is that the organic phosphate insecticides have an ovicidal effect which DDT does not have. The results with methoxychlor make the first explanation more probable.

Rhopobota naevana Hubner has been recorded feeding on fruit trees, holly, *Crataegus* and *Vaccinium* in Europe (Dampf 1908, Meyrick 1927). In the United States it has been recorded only from *Vaccinium* and is known as the black-headed fireworm. A *Vaccinium*-feeding form occurs in Europe and was for a time distinguished as *R. geminana*. Dampf (1908), on the basis of studies of the genitalia of these forms showed that this distinction was not justified. Although *R. naevana* feeds on holly in Europe, a form specific to holly, the holly bud moth, has been recorded only from North America. The holly bud moth was described as *Epinotia ilicifoliana* by Kearfott (1907). Heinrich (1923) considered *E. ilicifoliana* a sub-

[1] Approved for publication as technical paper, number 1076, Oregon Agricultural Experiment Station. Accepted for publication August 30, 1957.

species of *R. naevana* and it is at present determined as *R. naevana ilicifoliana* by Dr. J. F. Gates Clarke, although McDunnough (1939) lists *ilicifoliana* as a synonym of *naevana*.

The holly bud moth appears to have been introduced into British Columbia and to have subsequently spread southward. Heinrich (1923) lists specimens from British Columbia and Gibson (1928) indicates that it was a well-established pest in British Columbia in 1928. It was present in Washington in 1930 (Baker 1930). Schuh & Mote (1940) stated that the holly bud moth was first reported in Oregon in 1935, presumably in the Astoria region. The moth was not found in any other area of Oregon for a number of years but by 1955 it was a problem in holly orchards in the northern part of the Willamette Valley.

Oil-nicotine sprays have been used for holly bud moth control and have been applied in early spring before the buds begin to open. Inconsistent results have been obtained, however, and an added disadvantage has been the frequent difficulty of getting spray equipment into holly orchards as early in the season as necessary for proper timing of these sprays. These factors, in combination with the increased importance of the holly bud moth in Oregon holly orchards, led to the evaluation of other insecticides for holly bud moth control.

METHODS.—The holly bud moth overwinters in the egg stage. The eggs begin to hatch in the spring when the buds open and the new leaves appear. The larvae feed in the new growth. Insecticide applications were made between the time of hatching of the eggs and the time of blossoming of the holly. This is usually a 10- to 14-day period. Application of insecticides during blossoming is undesirable and, after blossoming, the larvae are within the rolled or webbed leaves, making control difficult.

All insecticides evaluated were applied with a hand spray gun. About 3 gallons of spray per tree were required for trees 8 to 10 feet tall which had not been pruned to obtain cut holly for the florist market. The amount of spray required per acre varied because no one system of spacing trees prevails in all holly orchards. In addition, the tree size varies, not only in relation to age, but also because of variety differences and the amount of wood removed when pruning to obtain cut holly. Wettable powder formulations were used in all cases. Four liquid ounces of Triton B-1956 per 100 gallons of water were added to all sprays because of the extreme smoothness of the holly leaves. The insecticides included and the rates per 100 gallons of water were as follows:

Malathion	4 lbs. 25% w.p.
Parathion	1 lb. 25% w.p.
DDT	2 lbs. 50% w.p.
Methoxychlor	3 lbs. 50% w.p.
Chlorthion	4 lbs. 25% w.p.
Diazinon	4 lbs. 25% w.p.

DDT and malathion were also used at the foregoing rates when applied in combination.

The plots were arranged in a randomized block design in all the experiments. Records were obtained by counting the number of infested tips on the trees from the lowest branches up to eye-level. These counts were made about 6 weeks after the insecticide application when the infested tips could be seen easily.

Table 1.—Evaluation of insecticides for holly bud moth control near Eagle Creek, Oregon in 1956.

INSECTICIDE	DATE APPLIED	INFESTED TIPS PER TREE[a]	PER CENT CONTROL[b]
Chlorthion	May 9	0.05	99.9
DDT	May 9	19.8	64.2
Diazinon	May 9	0.1	99.8
Malathion	May 9	None	100.
Malathion—DDT	May 1	0.2	99.6
Malathion—DDT	May 9	0.2	99.6
Methoxychlor	May 9	3.1	94.4
Parathion	May 1	0.4	99.3
Parathion	May 9	0.05	99.9
Check A	—	62.2	—
Check B	—	47.7	—

[a] Based on 16 trees (four trees per plot—four replicates).
[b] Based on mean of two checks.

RESULTS.—The first set of evaluations was made at Eagle Creek, Oregon in 1956. This area is east of the Willamette Valley toward Mt. Hood and the higher elevation results in phenological events occurring about 2 weeks later than in the Willamette Valley. Consequently, the dates of insecticide applications are not typical of Oregon's principal holly-growing areas. The May 9 application was made just before the blossoms opened. The results, presented in table 1, are typified by a high degree of control with all the organic phosphates, regardless of the date of application, by good control with methoxychlor, and by poor control with DDT.

Evaluations were restricted primarily to malathion and DDT in 1957. The objects of these tests were to confirm the efficacy of malathion, to better understand the timing of insecticide applications, and to get more information on the use of DDT for holly bud moth control. Three sets of evaluations were made in 1957. The first of these was near Wilsonville, Oregon. Malathion was applied April 25 and April 29. On April 25, the new growth varied from trees on which the leaf buds were just opening to trees with new leaves about $\frac{1}{4}$ inch long. On April 29, the new leaves on most of the trees were $\frac{1}{4}$ to $\frac{1}{2}$ inch long. Results are presented in table 2. The infestation in this planting proved to be light. Good control was obtained with all sprays.

Another set of evaluations was made near Astoria, Oregon. These plots were in an experiment station holly variety planting and there was considerable variation in the stage of development of the new growth. Approximately one-third of the trees had new leaves about $\frac{1}{4}$ to $\frac{1}{2}$ inch long. On most of the rest of the trees, the buds had just opened and the new growth was just starting. Leaf

Table 2.—Evaluation of insecticides for holly bud moth control near Wilsonville, Oregon. 1957.

INSECTICIDE	DATE APPLIED	INFESTED TIPS PER TREE[a]	PER CENT CONTROL
Malathion	April 25	None	100
Malathion	April 29	None	100
DDT	April 29	0.4	96
Check	—	9.0	—

[a] Based on 12 trees (three trees per plot—four replicates).

buds had not yet opened on a few trees. Of all the evaluations made, the time of these applications was the earliest in relation to the development of the new growth of the holly. The results are presented in table 3 and are characterized by a high degree of control with malathion and poor control with DDT.

Evaluations of insecticides for holly bud moth control were made near Eagle Creek again in 1957. The time of application was just before blossoming. Of all the evaluations made, these were the latest in relation to the development of the new growth of the holly. Results are presented in table 4. A high degree of control was obtained with malathion and good control was again obtained with methoxychlor. The effectiveness of DDT in these plots was surprising, in view of the severeness of the infestation. The only other test where good results were obtained with DDT was characterized by a light infestation.

The data in tables 1 and 4 indicate that methoxychlor is an effective insecticide for holly bud moth control when applied just before blossoming. The data in these two tables were analyzed to determine if the differences between methoxychlor and the phosphate insecticides were significant. Check plots were omitted from the analyses, as were the DDT plots in table 1. Both sets of data were transformed to the square root of $\frac{1}{2}+x$ before analysis of variance (Goulden 1952, p. 98). Estimates of significance were obtained by Duncan's (1955) multiple range test. All the organic phosphates and the malathion-DDT combinations in table 1 were significantly better than methoxychlor at the 5% level. Malathion was significantly better than methoxychlor at the 5% level in the plots represented in table 4.

DISCUSSION.—Eggs of the holly bud moth begin to hatch about the time the leaf buds open and the new growth begins. They do not, however, all hatch at once and some may not hatch until the blossoming period is in progress. The principal question that remains to be answered as a result of the investigations reported herein is why one application of DDT, made between the time of leaf bud opening and blossoming time, did not give consistently good control whereas malathion, or other organic phosphate insecticides, consistently gave a high degree of control when applied under the same conditions.

One possible explanation for the difference between malathion and DDT is that the holly bud moth may have some degree of natural tolerance to DDT. The fact that DDT sometimes gives very good control does not rule out this possibility. For example, the red-banded leaf roller (*Argyrotaenia velutinana* (Wlk.)) possesses a measure of natural DDT tolerance (Sternburg & Kearns 1952). Nevertheless, field evaluations of DDT for control of this insect give results varying from poor control to very good control (Glass & Chapman 1952).

Table 3.—Evaluation of insecticides applied May 1, 1957, for holly bud moth control near Astoria, Oregon.

INSECTICIDE	INFESTED TIPS PER TREE[a]	PER CENT CONTROL
DDT	45.7	50.2
Malathion A	3.3	96.4
Malathion B	2.2	97.6
Check	91.8	—

[a] Based on 12 trees (four trees per plot—three replicates).

Table 4.—Evaluation of insecticides applied May 10, 1957, for control of holly bud moth near Eagle Creek.

INSECTICIDE	INFESTED TIPS PER TREE[a]	PER CENT CONTROL
Methoxychlor	6.6	95.5
Malathion A	0.4	99.7
Malathion B	0.8	99.5
DDT A	15.1	89.8
DDT B	12.4	91.6
Check	148.1	—

[a] Based on 16 trees (four trees per plot—four replicates).

At the time malathion and the other organic phosphates were applied in the various tests, not all of the bud moth eggs would have hatched. Table 1 shows, however, that when parathion was applied on May 1, the control obtained was as good as when it was applied on May 9. A high degree of control was obtained even when malathion was applied at the earliest date in relation to the development of the holly (table 4). The effectiveness of single applications of the organic phosphate insecticides and the lack of consistent control with DDT could be explained by the assumption that the organic phosphates killed all the larvae which had hatched as well as those which had not hatched whereas DDT killed only those larvae which had hatched at the time of application. Parathion is known to be toxic to the eggs of some insects and work of this nature has been reviewed by Staudenmayer (1953). Good control was obtained consistently with methoxychlor which would not be expected to have an ovicidal effect. Consequently, natural tolerance to DDT would probably be the better explanation for the poor results obtained with this insecticide.

The larvae of certain other Lepidoptera feed on holly. The oblique-banded leaf roller (*Archips rosaceana* (Harr.)) has been reported as a pest of holly (Schuh & Mote 1940). Recently, *Archips rosana* L.[2], known in the West as the filbert leaf roller, has been reared from holly. Both these species feed in the new growth and cause injury similar to that of the holly bud moth. Larvae of the orange tortrix (*Argyrotaenia citrana* (Fern.))[2] were found feeding on holly berries in late summer and early fall but this insect has not as yet proven to be common or of economic significance in holly.

[2] The writer is indebted to Dr. J. F. Gates Clarke for determination of this species.

REFERENCES CITED

Baker, W. S. 1930. Holly insects. Proc. Wash. Hort. Soc. 26: 183–4.

Dampf, A. 1908. Ueber den Genialapparat von *Rhopobota naevana* Hb. (Lepid., Tortricid.) nebst Bemerkungen zur Systematik der Olethreutinae. Deutsche Entomologische Zeitschr. Iris 21: 304–29.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1–42.

Gibson, A. 1928. Insects of the flower garden and their control. Canada Dept. Agric. Bull. 99 (n.s.).

Glass, E. H., and P. J. Chapman. 1952. The red-banded leaf roller and its control. New York Agric. Expt. Sta. Bull. 755.

Goulden, C. H. 1952. Methods of Statistical Analysis. 2nd

Edition. John Wiley and Sons, N. Y.

Heinrich, C. 1923. Revision of the North American moths of the subfamily Eucosminae of the family Olethreutidae. U. S. Natl. Mus. Bull. 123.

Kearfott, W. D. 1907. Macrolepidoptera from the Black Mountain region of North Carolina, with descriptions of new species. Bull. Amer. Mus. Nat. Hist. 23: 153–67.

McDunnough, J. 1939. Check list of the Lepidoptera of Canada and the United States of America. Part II. Microlepidoptera. Mem. Southern California Acad. Sci. 2: 1–171.

Meyrick, E. 1927. A Revised Handbook of British Lepidoptera. Watkins and Doncaster, London.

Schuh, J., and D. C. Mote. 1940. Insect pests of holly. Oregon Agric. Expt. Sta. Circ. Inform. 228.

Staudenmayer, T. 1953. The influence of "E 605" on the respiration of silkworm eggs. Hofchen-Briefe 6: 159–66 (English Edition).

Sternburg, J., and C. W. Kearns. 1952. Metabolic fate of DDT when applied to certain naturally tolerant insects. Jour. Econ. Ent. 45: 497–505.

Mealybugs Attacking Citrus in California—A Survey of Their Natural Enemies and the Release of New Parasites and Predators[1]

B. R. BARTLETT[2] and D. C. LLOYD,[3,4] *University of California Citrus Experiment Station, Riverside*

ABSTRACT

From 1953 through 1955 a survey was made of the natural enemies of the mealybugs attacking citrus in California. *Pseudococcus citri* (Risso), the most destructive mealybug on citrus in California, was most abundant in the intermediate citrus areas or zones of moderate humidity. *Pseudococcus maritimus* (Ehrh.), closely rivaling *P. citri* in severity on citrus, requires high humidity for optimum development. Its distribution overlaps that of *P. citri* in the intermediate zone and that of *Pseudococcus adonidum* (L.), which was damaging to citrus in the more humid coastal areas. *Pseudococcus gahani* Green is rare. It overlaps the three other species in its distribution.

Cryptolaemus montrouzieri Muls. was in general the most efficient predator of heavy infestations of mealybugs on citrus. However, *Sympherobius* spp. and *Chrysopa* spp. are sometimes major factors in reducing heavy populations in certain areas. *Leucopis bella* Loew, *Coccinella californica* Mann., *Hyperaspis lateralis* Muls., and *Scymnus sordidus* Horn are less efficient mealybug predators in the order named. The relative abundance of the different parasites permanently established on each of the four citrus-infesting mealybugs is shown in a table along with the stages of host attacked. Some of these parasites attack a number of mealybug species, but always select the same host stages. A synoptic list of 19 species of mealybug-feeding natural enemies imported into California within the past 10 years is presented along with their host preferences and stages of host attack. Of the newly imported natural enemies, *Allotropa citri* Mues. and *Anagyrus pseudococci* Gir. have been recovered from field release sites.

From 1953 through 1955 an extensive survey of the relationship of natural enemies to mealybugs on citrus was made by the Department of Biological Control of the University of California at Riverside. This field examination was made in connection with the release and attempted recovery of a number of newly imported parasites and predators of mealybugs. During the same period a comprehensive study was carried out in the Fontana, California, laboratory of the Commonwealth Institute of Biological Control on host preferences and host stages attacked by the mealybug parasites. The information obtained from these cooperative studies is presented as a basis for further efforts toward biological control of mealybugs on citrus.

DISTRIBUTION AND DEVELOPMENT OF MEALYBUGS ATTACKING CITRUS.—Four species of mealybugs are commonly found on citrus in California. *Pseudococcus citri* (Risso), the citrus mealybug, is the most widespread and causes the greatest amount of damage. This species is closely rivaled in severity by *Pseudococcus maritimus* (Ehrh.), the grape mealybug, commonly referred to on citrus as Baker's mealybug. The long-tailed mealybug, *Pseudococcus adonidum* (L.) [*longispinus* (Targ.)], in localized areas frequently reaches damaging proportions, but outbreaks of this species are most often related to a disturbance of biological control by ants, insecticides, or heavy road dust residues. The citrophilus mealybug, *Pseudococcus gahani* Green, is rare in most citrus areas, being kept under nearly perfect biological control by its extremely efficient parasites (Compere & Smith 1932).

There is considerable overlap in geographical distribution of the four species of mealybugs. The predominance of each at any single location is related to optimum humidity requirements for the species. The longtailed mealybug, largely coexistent on citrus with Baker's mealybug, is confined to the most coastal humid areas, where it is often abundant on citrus or other plant hosts whose succulence or growth habits supply a moist environment.

The distribution of Baker's mealybug overlaps to some degree that of both the long-tailed and the citrus mealybug on citrus. It is most severe as a pest in the humid coastal areas, but is of fairly common occurrence in the intermediate citrus-producing districts. The citrus-infesting form of *Pseudococcus maritimus* does not appear to be exactly similar to the form which occurs on grapes in the more inland areas of the State. Although indistin-

[1] Paper No. 981, University of California Citrus Experiment Station, Riverside. Accepted for publication August 30, 1957.

[2] Associate Entomologist in the Department of Biological Control.

[3] Research Associate, Department of Biological Control, University of California Citrus Experiment Station, Riverside, and Entomologist in the Commonwealth Institute of Biological Control at Fontana, California.

[4] The authors acknowledge their indebtedness in the release programs to Mr. W. L. Worthy of the Oxnard Pest Control Association, Mr. W. C. Beckley of the Santa Paula Associates, and Mr. H. B. Lorbeer, of the Fillmore Citrus Protective League.

guishable taxonomically, these forms support different parasitic fauna and inhabit different climatic areas.

The citrus mealybug is generally distributed in the so-called intermediate citrus belt, but at times it is abundant in the coastal areas. This mealybug appears to be less influenced by aridity extremes than any of the other species named above. Only rarely, however, is this species encountered in the inland citrus-producing areas.

Of the mealy bugs attacking citrus in California, the citrophilus mealybug is of particular interest. Judging from distributional records preceding the establishment of its very efficient internal parasites, the climatic range of this species overlapped that of all the other citrus-infesting mealybugs.

In the field, on citrus, each of the mealybug species has four to five overlapping broods. In the laboratory, where these four species of mealybugs have been reared upon sprouted potato tubers at 80° F., the stages of *Pseudococcus citri* have developed as follows: the first instar lasts approximately 5 days; the second an additional 4 days; the third stage approximately 6 days; and in the fourth stage, egg production occurs approximately 2 weeks after the last molt. The periods required for development of *P. maritimus*, *P. adonidum*, and *P. gahani* are somewhat longer than that for *P. citri*, increasing in the order named. The very small size of the early instars, in comparison with the size attained in the last stage of mealybug development, is particularly important with respect to parasite attack, since field recognition of the parasite species may, at times, be based upon the size range of attacked hosts. The average length of specimens of *P. citri* at the molts under laboratory conditions has been determined as follows: first molt approximately 0.5 mm., second molt 1.1 mm., third molt 1.5 mm. The length at maturity may be 1.6 to 3.5 mm.

ESTABLISHED MEALYBUG PREDATORS.—Except with *Pseudococcus gahani* and to some extent *P. adonidum*, the ultimate reduction of dense mealybug populations on citrus is usually dependent upon the action of predators rather than of parasites. The predators of mealybugs are undependable with regard to their occurrence, and are variable in their distribution from one area to another. With very few exceptions, however, the predators show little discrimination between mealybug hosts. It is quite possible to rate the relative efficiency of the mealybug predators for any single district, but difficult to rank their efficiency over the general mealybug-infested citrus area.

In considering the principal predators individually we must conclude that the ladybird beetle *Cryptolaemus montrouzieri* Muls. more often than any other species is capable of reducing heavy mealybug infestations on citrus. An evaluation of the effectiveness of naturally occurring populations of this predator is confused by the general practice of seeding mealybug-infested orchards with insectary-reared adult ladybird beetles (Smith & Armitage 1931). Periodic releases of insectary-reared stocks of *Cryptolaemus* are frequently followed by outstanding control of mixed mealybug infestations. However, a similar control by naturally developing *Cryptolaemus* populations often occurs in non-seeded orchards, that is those in which no periodic liberations were made. Surveys of closely associated *Cryptolaemus*-seeded and non-seeded citrus groves by the authors leads them to the view that, more often than not, considerable benefit results from seeding, this benefit extending to nearby non-seeded areas. The practice has appeared to be particularly valuable following extremely cold winters in which the natural *Cryptolaemus* population has been much reduced. Although this predator is not always dependable in its occurrence, it is the most efficient predator if climatic and environmental conditions are suitable. If the conditions are not suitable, even releases of large numbers of insectary-reared adults give ineffective results.

In an overall assessment of mealybug predators, the brown lacewings (*Symperobius* spp.) may well be ranked next to *Cryptolaemus* in effectiveness. The abundance of these lacewings varies greatly with locality. They predominate in the coastal areas of Orange and San Diego counties and are generally responsible for the seasonal spring decline in populations of all species of mealybugs in those areas. The natural abundance and usefulness of this predator, however, in the Ventura County citrus-producing districts is much less.

Green lacewings (*Chrysopa* sp.) which may be ranked third in overall efficiency as mealybug predators, are very effective in Orange and San Diego counties, but are of much more limited influence in Ventura County.

The other predators of mealybugs on citrus in California are of limited value. The dipteran *Leucopis bella* Loew sometimes becomes abundant feeding in the egg sacks of Baker's and citrus mealybugs. Field samples have indicated this predator to be principally a high-density feeder, particularly inefficient in low host populations. *Coccinella californica* Mann. sometimes moves in to decrease heavy mealybug infestations on citrus when aphid hosts become scarce on the native vegetation in the summer. The coccinellid, *Hyperaspis lateralis* Muls., becomes abundant in certain localized orchards as an overflow from the cypress windbreaks, where it feeds on related coccids. *Scymnus sordidus* Horn is rare and relatively ineffective.

ESTABLISHED MEALYBUG PARASITES.—Parasites play an important part in mealybug control on citrus in California. It is the opinion of the authors that, in general, their influence is principally as a restraint on low-density mealybug populations rather than as a control of heavy infestations. Parasites, except those of the citrophilus mealybug and to a lesser extent those of the long-tailed mealybug, are generally not efficient enough to prevent occasional pest outbreaks. The restraint on increase of long-tailed mealybugs by parasites has been indicated by the resulting population increases that occur when parasites are eliminated either through insecticidal check methods (DeBach 1946) or by means of ants (DeBach *et al.* 1951).

A synoptic list of the principal parasites of the mealybug species attacking citrus in California is presented in table 1 in conjunction with the stage of host attack as determined by comparative laboratory tests. The ranking of relative abundance given the parasites is necessarily an arbitrary one, based principally on field sampling, but weighted to a considerable extent by published and unpublished information upon field and laboratory studies with selected host material. The relative abundance of the parasites of *Pseudococcus adonidum* is essentially that

Table 1.—Principal parasites established on four species of mealybugs attacking citrus in California.

Parasite Species	Mealybug Stage Attacked				Alphabetical Ranking of Relative Abundance of Parasites Found Attacking *Pseudococcus* on Citrus			
	1st	2d	3d	4th	*citri*	*maritimus*	*adonidum*	*gahani*
Anarhopus sydneyensis Timb.		X	X	X			A	
Chrysoplatycerus splendens (How.)		X	X	X	E	A	F	E
Coccophagus gurneyi Comp.		X	X	X		B	C	A
Leptomastidea abnormis (Gir.)	X	X	X		A			F
Leptomastix dactylopii How.		X	X	X	B			
Pauridia peregrina Timb.	X	X	X		C			
Pseudaphycus angelicus (How.)	X	X	X		D		E	D
Tetracnemus peregrinus Comp.	X	X	X			D	D	C
Tetracnemus pretiosus Timb.	X	X	X			C	B[a]	B
Zarhopalus sheldoni Ash.			X	X		E		

[a] Attempts to rear *T. pretiosus* from this host on potatoes, lemons, grapefruit, and *Citrullus* melons in the laboratory have been unsuccessful.

listed by DeBach (1949). The relative abundance of the parasites of *Pseudococcus gahani* has been inferred in a large part from circumstantial evidence, since the host is held to such a low density as to be of rare occurrence on citrus in southern California. Where laboratory specimens reared on potato sprouts have been placed in the field for attraction and oviposition of parasites, *Tetracnemus pretiosus* Timb., *Coccophagus gurneyi* Comp., and *Pseudaphycus angelicus* (How.) have been recovered in nearly equal numbers. Contamination by the long-tailed mealybug is known to have occurred on these stocks, however. The rapid control of *P. gahani* after the initial releases of *T. pretiosus* and *C. gurneyi* indicated the efficiency of both, and the particular ability of the latter species rapidly to overtake high-density host populations (Compere & Smith 1932). The ability of both of these species to survive on other mealybug hosts has no doubt contributed greatly to their amazing ability to restrain *P. gahani* increase even where drift insecticides have caused upsets in the other mealybug species. Prior to the introduction of *T. pretiosus* and *C. gurneyi* to California, records indicated that *P. angelicus* was relatively ineffective against *P. gahani*, so the extraordinary reduction by *C. gurneyi* and *T. pretiosus* was clearly defined.

THE IMPORTATION AND RELEASE OF NEW NATURAL ENEMIES ATTACKING MEALYBUGS ON CITRUS.—An examination of the field-collected survey samples and published records on the natural enemies of mealybugs attacking citrus in California discloses that satisfactory biological control has not been achieved for either *Pseudococcus citri* or *P. maritimus* on this host plant. Since 1927, when an active campaign was launched to control *P. gahani* by imported natural enemies, no further concentrated efforts have been made to augment the biological control of either *P. citri* or *P. maritimus*. The problem has in the intervening period become more acute, with the subsequent raising of fruit and production standards.

During the past decade there have been some opportunities to procure mealybug natural enemies. Some were obtained from tropical areas and were consequently probably not suited to California climatic extremes. A few were associated with other mealybug host species in their native homes. An annotated list of these species and

Table 2.—New natural enemies imported and released against four species of mealybugs attacking citrus in California.

Species	Country of Origin	Collector	Year Import	*Pseudococcus* Attacked and Alphabetical Ranking of the Host Preference of the Natural Enemies[a]				Mealybug Stage Attacked				Approximate Number of Parasites Released
				citri	*adonidum*	*maritimus*	*gahani*	1st	2d	3d	4th	
Parasites												
Acroaspidia myrmicoides Comp. & Zinna	Trinidad	Bennett	1954	D	C	A	B	X	X	X	X	5,000
Allotropa citri Mues.	S. China	Gressitt	1950	A	B			X	X			50,000
Anagyrus kivuensis Comp.	Kenya	Compere	1948	A							X	12,000
Anagyrus pseudococci Gir.	Brazil	Lloyd	1953	A	C	D	B		X	X	X	20,000
Anagyrus sp. (near *pseudococci*)	Italy	Flanders	1955	A	C	D	B		X	X	X	10,000
Bothriocraera bicolor Comp. & Zinna	Trinidad	Simmonds	1953		C	A	B		X	X	X	20,000
Blepyrus saccharicola Gahan	S. U.S.	Lloyd	1952	D	A	C	B		X	X	X	200
Leptomastidea sp. (near *abnormis* Gir.)	Mexico	DeBach	1956	A			B	X	X	X		30
Pseudaphycus perdignus Comp. & Zinna	Eritrea	van den Bosch	1953	A				X	X	X	X	500,000
Tropidophryne melvillei Comp.	Kenya	Compere	1948	A							X	2,000
Predators												
Brumus suturalis (Fabr.)	India	Angalet	1955	A	A	A	A	X	X	X	X	1,000
Chilocorus angolensis Crotch	Kenya	Compere	1948	A	A	A	A	X	X	X	X	700
Coccinellid (near *Platynaspis*)	Eritrea	van den Bosch	1953	A	A	A	A	X	X	X	X	2,000
Exochomus flavipes (Thunberg)	Kenya	Compere	1948	A	A	A	A	X	X	X	X	100
Hyperaspis jucunda Muls.	Trinidad	Bennett	1955	A	A	A	A	X	X	X	X	150
Hyperaspis sp. (near *globula* Casey)	Mexico	DeBach	1954	A	A	A	A	X	X	X	X	3,000
Hyperaspis sp.	Eritrea	van den Bosch	1953	A	A	A	A	X	X	X	X	1,200
Nephus sp.	Trinidad	Bennett	1955	A	A	A	A	X	X	X	X	100
Scymnus quadrivittatus Muls.	Kenya	Compere	1948	A	A	A	A	X	X	X	X	4,000

[a] As established by laboratory tests.

their host preferences and stages of attack, as presented in table 2, represents a joint effort, in most instances, by the Department of Biological Control of the University of California and the Commonwealth Institute of Biological Control. Many of the species listed were obtained specifically for the Commonwealth Institute in its campaign against the cacao-infesting mealybugs so devastating to the Gold Coast cacao plantations of Africa and were only incidentally reared and tested for possible use in California citrus orchards. The larger releases, as shown in table 2, resulted from cooperative rearing programs with three grower-operated commercial insectaries and represent the combined releases of all agencies.

To date, only the species *Allotropa citri* Mues. and *Anagyrus pseudococci* Gir. have been recovered from release sites.

REFERENCES CITED

Compere, Harold, and Harry S. Smith. 1932. The control of the citrophilus mealybug, *Pseudococcus gahani*, by Australian parasites. Hilgardia **6**(17): 585–618.

DeBach, Paul. 1946. An insecticidal check method for measuring the efficacy of entomophagous insects. Jour. Econ. Ent. **39**(6): 695–7.

DeBach, Paul. 1949. Population studies of the long-tailed mealybug and its natural enemies on citrus trees in southern California, 1946. Ecology **30**(1): 14–25.

DeBach, Paul, C. A. Fleschner, and E. J. Dietrick. 1951. A biological check method for evaluating the effectiveness of entomophagous insects. Jour. Econ. Ent. **44**(5): 763–6.

Smith, Harry S., and H. M. Armitage. 1931. The biological control of mealybugs attacking citrus. California Agric. Expt. Sta. Bull. **509**: 1–74.

The Effects of Temperature on the Efficiency of Insecticides Applied Topically to Boll Weevils Differing in Susceptibility to Chlorinated Hydrocarbon Insecticides[1]

M. R. YOUNG and J. S. ROUSSEL, *Louisiana Agricultural Experiment Station, Baton Rouge*

ABSTRACT

Greater mortality of the boll weevil (*Anthonomus grandis* Boh.) resulted at the higher rather than at the lower extreme of the post-treatment range of temperatures with one exception—the response of the resistant strain to the endrin-malathion combination wherein greater mortality resulted at the lower extreme of the temperature range.

The two strains (resistant and susceptible or reference) differed greatly in their susceptibility to the chlorinated hydrocarbons. Toxaphene and endrin were ineffective against the resistant strain.

Endrin and malathion, when combined, were antagonistic at 90° F., but showed a synergistic effect at 60° and 80° F. On the other hand, toxaphene and malathion combined were antagonistic at 60° F., but showed a synergistic effect at 80° and 90° F. The resistant and reference strains responded similarly to temperature changes for each insecticide used in respect to antagonistic or synergistic effects. These findings indicated that the mechanism which causes synergistic effect and antagonistic effect had differing temperature coefficients of action for the two combinations (endrin-malathion and toxaphene-malathion).

The fact that the same strain (either resistant or susceptible) responded differently to each of the three insecticides (endrin, toxaphene, and malathion) when held at different post-treatment temperatures, indicated that each insecticide had a different mode of action. The difference in the amount of insecticide required to control the reference strain as compared with the amount required to control the resistant strain, when held at varying post-treatment temperature levels, is further supporting evidence for such a conclusion.

This is the logical expectation in the case of malathion (an organic phosphate) and the chlorinated hydrocarbons as a group. However, it is noteworthy that endrin and toxaphene, both being chlorinated hydrocarbons, appeared to have different modes of action.

In addition to susceptibility to chlorinated hydrocarbons, there was an indication that the two strains differ in other physiological functions. The fact that more malathion was required to control the chlorinated-hydrocarbon-susceptible strain than was required to control the resistant strain prevents the assumption that the susceptibility to malathion and the susceptibility to endrin or toxaphene was controlled by the same mechanism.

It is also significant that although endrin was relatively nontoxic to the resistant strain, it still influenced the toxicity of malathion to this strain.

It is known that temperature has an effect upon the toxic action of insecticides. The means by which it exhibits this effect is not definitely known or at least not agreed upon. At present no general rule can be applied to the relationship between toxic action and temperature. Certain insecticides give increased kill as temperature increases (positive temperature coefficient) while others give greater kill as temperatures decrease (negative temperature coefficient). Each insecticide seems to have its own peculiarities.

The primary purpose of this study was to compare the response of a chlorinated hydrocarbon resistant strain and a chlorinated hydrocarbon susceptible strain of boll weevil, *Anthonomus grandis* Boh., when treated topically with certain insecticides and held at different temperature levels. A second objective was to determine the difference in response of the two strains to organic phosphate insecticides, chlorinated hydrocarbon insecticides and combinations of the two.

REVIEW OF LITERATURE.—Hoffman & Lindquist (1949) found that DDT, methoxychlor, and DDD displayed negative temperature coefficients for speed of knockdown and mortality of the house fly, *Musca domestica* L., when flies were exposed at 70° and 90° F. with humidities of 40 to 60%. Heptachlor, parathion, and

[1] Accepted for publication August 30, 1957.

toxaphene showed positive temperature coefficients, as did malathion for Rai *et al.* (1956).

The greatest difference in the effect of DDT, pyrethrin, lauryl thiocyanate, nicotine, and dinitro-o-creosol when applied topically to the red flour beetle, *Tribolium castaneum* (Hbst.), was produced by the temperature after treatment, higher mortality occurring at the lower temperature levels (Potter & Gillham 1946). They concluded that the probable reason for increased mortality under lower temperatures after treatment was due to the physiological conditioning of the insect and not to the effect of temperature on the poison or the medium in which it was carried.

Dustan (1947) found DDT to have a negative temperature coefficient to larvae of the diamondback moth, *Plutella maculipennis* (Curt.), when applied as a contact spray or when taken orally. Pradham (1949) showed that when red flour beetles were exposed to films of DDT at identical temperatures, but held at different post-treatment temperatures that mortality was higher at the lower holding temperature. Exposure at different temperature levels with the same holding temperature resulted in greater mortality occurring at the higher temperature of exposure.

A negative temperature coefficient was observed for the large milkweed bug, *Oncopeltus faciatus* (Dall.), when injected with DDT and held at post-treatment temperatures of 10°, 15° 22°, and 29° C. by Woodruff (1950). Contrasting results were obtained by Fan *et al.* (1948), with larvae of the yellow-fever mosquito, *Aedes aegypti* (L.), and midge larvae, *Chaoborus sp.* treated with DDT by immersion and injection methods.

Vinson & Kearns (1952) showed that DDT had a negative temperature co-efficient when applied topically or injected into female American cockroaches, *Periplaneta americana* (L.). Chemical analysis correlated with onset of toxic symptoms from topical applications showed that DDT penetrated faster at 35° than at 15° C. However the change of equilibrium between the opposing forces of penetration and detoxification of DDT brought about by temperature manipulation did not account for the negative temperature coefficient of action. Percentage of absorbed DDT which was metabolized was approximately the same at the high or low temperatures when equivalent dosages were compared. They concluded that the direct effect of temperature on the metabolism of injected DDT was incidental to the intoxicating process. Thus the negative temperature coefficient of action of DDT must be based on the effect of temperature on the susceptibility of some physiological system to DDT. Munson *et al.* (1954) found that the holding capacity of the lipids from American cockroaches for DDT and methoxychlor was greater at the higher extremes of the temperature range. They also found that bromine-DDT, fluorine-DDT, and methoxychlor resulted in negative temperature coefficients. A negative temperature coefficient was exhibited by chlordane except for lower doses at higher temperature levels when applied to saw-toothed grain beetles, *Oryzaephilus surinamensis* (L.).

Lofgren & Cutkomp (1956) showed that cockroaches on high protein diets were more sensitive to high temperatures and were more susceptible to DDT at both high and low post-treatment temperatures (15° and 30° C.) than when on high carbohydrate diets. All three diets produced negative temperature coefficients. When the lipid content of the cockroaches was high, more DDT was required for kill.

Guthrie (1950) reported that DDT, pyrethrin, and lindane gave negative and aldrin and dieldrin positive temperature coefficients on the German cockroach, *Blattella germanica* (L.).

When pyrethrin was applied as a spray in an oil solution, mortality of the beet leafhopper, *Circulifer tenellus* (Baker), was increased by raising the temperature at time of application, but was increased to a greater extent by lowering the temperature after application (Harries *et al.* 1945).

Menn *et al.* (1957) found that when resistant house flies were exposed to larval diets which included 25 p.p.m. and 2,000 p.p.m. DDT there were small amounts of unchanged DDT in adult flies from both diets. However, 30% mortality resulted from the high concentration. Mortality could not be correlated with the amount of unchanged DDT present in the adults; rather, it was probably caused by a synergistic effect of DDE with small amount of unchanged DDT present. Response of susceptible flies to toxic action resulting from lowered temperature was faster than for resistant ones.

Hurst (1949) found that blowfly larvae, *Calliphora erythrocephala*, exposed to crystalline DDT at 20° C. were very resistant. When the temperature was raised to 36° C. for 2 hours and then lowered to 20° C. they became paralyzed, but recovered again at 36° C. This indicates that permeability increased with increased temperature. Ellisor & Blair (1940) investigated the effects of temperature on the toxicity of inorganic stomach poisons to the larvae of the southern armyworm, *Prodenia eridania* (Cram.), and the velvetbean caterpillar, *Anticarsia gemmatalis* Hbn. Calcium arsenate, acid lead arsenate, basic copper arsenate, and synthetic cryolite all showed negative temperature coefficients for both species tested, with the exception of synthetic cryolite for the velvetbean caterpillar, which gave the same mortality at both temperatures.

McIntosh (1954) determined temperature coefficients for saw-toothed grain beetles and red flour beetles killed with volatile solid insecticides. These were negative for BHC and DFDT but positive for aldrin. Since vapor pressure of BHC is affected more by a temperature increase than aldrin, vapor-pressure phenomena cannot explain the difference in temperature coefficients of the two insecticides.

Eagleson (1942) found that temperature changes altered toxicity effects less for house flies treated with lethane than with pyrethrin. At 38° C. lethane was more effective in contrast to pyrethrin which was more effective below 34° C. Both insecticides showed negative temperature coefficients.

Blum & Kearns (1956) found that pyrethrin had a negative temperature coefficient between 15°C. and 35°C. when applied to the American cockroach. By use of C^{14} labeled pyrethrin they determined that penetration of pyrethrin was more than twice as fast at 35° C. than it was at 15° C. Blood taken from poisoned cockroaches and injected into adult flesh flies, *Sarcophaga crassipalpus*, Macg., caused the flies to exhibit the same symptoms as

the cockroaches from which the blood came. However, the blood taken from the cockroaches was non-radioactive, indicating that the toxin in the blood was not pyrethrin. From these data it appears evident that at least a part of the toxic action of pyrethrin is due to its ability to stimulate production of a toxic substance in the cockroach.

In tests with the boll weevil relative to seasonal changes, Reiser *et al.* (1953) found that fat content increased as the season progressed and that weevils became increasingly harder to kill with toxaphene. They also found that surviving weevils were fatter than susceptible ones. However, this was also the case with the controls. Resistance to calcium arsenate also increased as the season progressed and fat increased. In view of these findings, they concluded that resistance is not directly related to fat content.

MATERIALS AND METHODS.—Insecticides used in the experiment were endrin, toxaphene, malathion, a combination of endrin and malathion, and a combination of toxaphene and malathion. Endrin and malathion were combined at the ratio of 2 parts to 5, respectively. Toxaphene and malathion were combined at the ratio of 4 parts to 1, respectively.

Chlorinated hydrocarbon resistant weevils used in this study were collected from a field at St. Joseph, La., and susceptible weevils were collected from a field at Baton Rouge, La. Susceptible weevils are hereinafter referred to as reference strain. Boll weevils were collected from each source at weekly intervals from September 12, 1956, to October 22, 1956. A large net approximately 36 inches in diameter was used for collecting. Terminal ends of cotton stalks were placed into the net and shaken causing the boll weevils to be dislodged and fall into the net. This operation was continued until a sizeable amount of trash was collected in the bottom of the net. The weevils were then removed from the net and put into 1-gallon ice cream cartons which were provided with cotton squares and blossoms as a food source. Holes were made in the tops to allow for air passage. Boll weevils were kept in these cartons and held under laboratory conditions until used. Fresh food was supplied each day when they were held for more than 24 hours.

A micrometer-driven syringe[2] was used for the application of insecticides. The insecticides were prepared as an acetone solution and serially diluted to concentrations necessary to obtain a range of mortalities suitable for plotting a dosage-mortality curve.

One-half pint jelly jars, with metal lids provided with holes for air exchange, were used for holding boll weevils after the application of insecticide. A total of 11,305 boll weevils was used in this study.

PROCEDURE.—Weevils were weighed in groups of 10 before the insecticide was applied. Weights were recorded to the nearest 0.1 milligram. After the weevils were weighed, 1 microliter of insecticide solution was applied to the dorsum of the metathoracic segment of individual weevils. The weevils were than placed (in groups of 10) in ½-pint jelly jars, as described above, with cotton squares provided for food. The jars were capped with perforated metal lids, thus permitting free air movement.

After the weevils were treated, the resistant and reference strains were each further divided into three sub-groups. One sub-group for each of the strains was placed in a room at 60° F. with a relative humidity of 70%; one was placed in a room at 80° F. with a relative humidity of 50%; and the third sub-group was placed in a cabinet at 90° F. in which the relative humidity was 50%.

Table 1.—LD-50's, at 48 and 72 hours, expressed in mg./gm. for reference and resistant strains held at 80° F. after treatment.

	REFERENCE STRAIN		RESISTANCE STRAIN	
INSECTICIDE	48 hrs.	72 hrs.	48 hrs.	72 hrs.
Toxaphene	114.90	7.35	1074.0	10.76
Endrin	0.18	0.01	2.47[a]	9.85[a]

[a] LD-50 at 72 hours is larger due to heterogeniety of mortality data resulting in a very flat dosage-mortality curve.

Mortality counts were made at 24, 48, and 72 hours. The criterion for death at 24 or 48 hours was failure to move after prodding the weevil with a pencil. Weevils were considered dead at 72 hours if unable to stand erect.

Per cent control was determined by use of Abbott's formula. LD-50's and LD-90's were computed by Finney's method (Finney 1952).

DISCUSSION OF RESULTS.—The differential response of the resistant and the reference strain of boll weevils to endrin and toxaphene is shown in table 1. The LD-50's for the resistant strain were considerably greater for endrin at each temperature than for the reference strain. The LD-50's for toxaphene were high in relation to endrin for both strains. The difference between strains was not as great for toxaphene as it was for endrin (table 1). Toxaphene was relatively ineffective against the boll weevil when compared with endrin in September and October. Results of cage tests by Rainwater & Gaines (1951) showed that the boll weevil became increasingly more difficult to kill as the season advanced into the fall months. They stated that by their method of testing, 240 times as much toxaphene was required to kill the same percentage of weevils in October as in July. They further stated that the ratio of the amount of insecticide required varied with different insecticides. It is believed that weevils used during the course of this experiment (September 13 to October 22) had already undergone such a seasonal change; however, records were not kept earlier in the season to verify such a belief.

Temperature variations had a greater effect on the efficiency of toxaphene than on endrin. At 60° F. the ratio of the amount of toxaphene to that of endrin required for an LD-50 was 1,476 to 1. At 80° F. the ratio was 668 to 1 and at 90° F., 356 to 1. It is noteworthy that these ratios are so high in view of the fact that field recommendations for toxaphene are only 10 times the amount of endrin, i.e., 2 pounds toxaphene to 0.2 pound endrin per acre. There is also a notable increase in the efficiency of toxaphene with increased temperature as compared with endrin.

A combination of data from a malathion treatment, endrin and malathion combined, and a combination of toxaphene with malathion indicated that malathion is more effective on the resistant strain than on the refer-

[2] Micrometer syringe was purchased from Burrought Wellcome and Co., London.

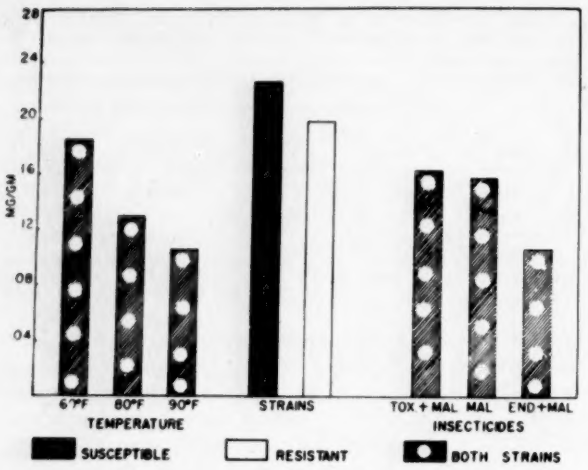


FIG. 1.—LD-50's in mg./gm. for combined data of malathion, endrin plus malathion, and toxaphene plus malathion computed on the basis of malathion alone.

ence strain (fig. 1). The LD-50's presented in figure 1 are computed on the basis of malathion alone. This was done because malathion was most likely the responsible agent for the mortality obtained in the resistant strain. In this way the LD-50's for the resistant and reference strains are on a comparable basis. All insecticides showed a positive temperature coefficient in the range of 60° to 90° F. with the exception of the endrin-malathion combination when applied to the resistant strain (table 2). This agrees with work by Hoffman & Lindquist (1949) which showed that heptachlor, dieldrin, and toxaphene had positive temperature coefficients in the range of 70° to 90° F. The ratio of LD-50's for the resistant strain to that of the reference strain decreased as the temperature increased from 60° to 90° F. for endrin and toxaphene. For example, at 60° F. the ratio between the reference and resistant strain for endrin was 1:10,572, whereas, at 90° F. the ratio had decreased to 1:35 (table 3). For malathion, endrin-malathion combination, and toxaphene-malathion combination the ratio increased as the temperature increased from 60° to 90° F.

The analysis of variance presented in table 4 for all

Table 2.—LD-50's at 72-hour observation expressed in mg./gm.

Insecticide[a]	Temperature, °F. 60	80	90
	Reference Strain		
Endrin	.0874	.0110	.0045
Endrin+malathion	.0240	.0124	.0127
Malathion	.0413	.0291	.0198
Toxaphene	129.9000[b]	7.3500	1.6380
Toxaphene+malathion	.0482	.0216	.0161
	Resistant Strain		
Endrin	964.3000[b]	9.85	.1630
Endrin+malathion	.0141	.0175	.0237
Malathion	.0242	.0258	.0173
Toxaphene	43,640.0000[b]	10.7600[b]	8.85
Toxaphene+malathion	.0341	.0233	.0166

[a] Combinations computed on the basis of malathion alone.
[b] LD-50's obtained by extrapolation.

Table 3.—Amount of insecticide required by the resistant strain for an LD-50 in comparison with that required by the reference strain with an index of 1 for the reference strain.

Insecticide[a]	Temperature, ° F. 60	80	90
Endrin	10,572.0000	895.40	35.43
Toxaphene	763.0000	1.46	5.46
Malathion	.5859	.87	.87
Toxaphene+malathion	.7075	1.08	1.08
Endrin+malathion	.5875	1.41	1.87

[a] LD-50's for treatments containing malathion were computed on the basis of malathion alone.

treatments which included malathion shows the significance of all factors and their interactions.

Brown (1951) considered two factors involved in the toxic action of an insecticide. They are listed as: (1) "The speed and degree to which the poison is taken up by the insect and acts upon its tissues" and (2) "The speed and degree to which the poison is eliminated by excretion, breakdown, or detoxification." He further proposed that with decisive doses of quick-acting poisons, it would be expected that mortality would be quicker and greater when application is made at a higher temperature than at a lower one. With marginal doses of a slow-acting poison the expectation is that recovery will be less at the lower range of temperature extreme than at the higher one, and that mortality will be greater although it develops at a slower rate. According to Brown, the air temperature when application is made decides the rate of uptake or the immediate toxicity, and the post-treatment air temperature governs the rate of recovery. Results of our study do not follow the pattern proposed by Brown. As previously mentioned, a positive temperature coefficient existed for all insecticides with only one exception. Since the temperature at the time of application was essentially the same (normal laboratory temperature) for all insecticides, mortality should have been greater at the lower holding temperature in order for these results to follow the reasoning of Brown. Data presented in figure 1 show that this was not the case.

The endrin-malathion combination was significantly more effective than malathion or the toxaphene-ma-

Table 4.—Analysis of variance for all treatments containing malathion. LD-50's computed on the basis of malathion alone.

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F-Ratio
Total	17	15.89		
Strains	1	0.45	0.45	19.56[a]
Temperature	2	5.21	2.60	113.04[b]
Insecticide	2	3.26	1.63	70.87[b]
Strain×Temp.	2	2.49	1.25	54.35[b]
Insecticide×Strain	2	0.72	0.36	15.65[a]
Temp.×Insecticide	4	3.67	0.92	40.00[a]
Error	4	0.09	0.023	

[a] Significant at .05 level.
[b] Significant at .01 level.

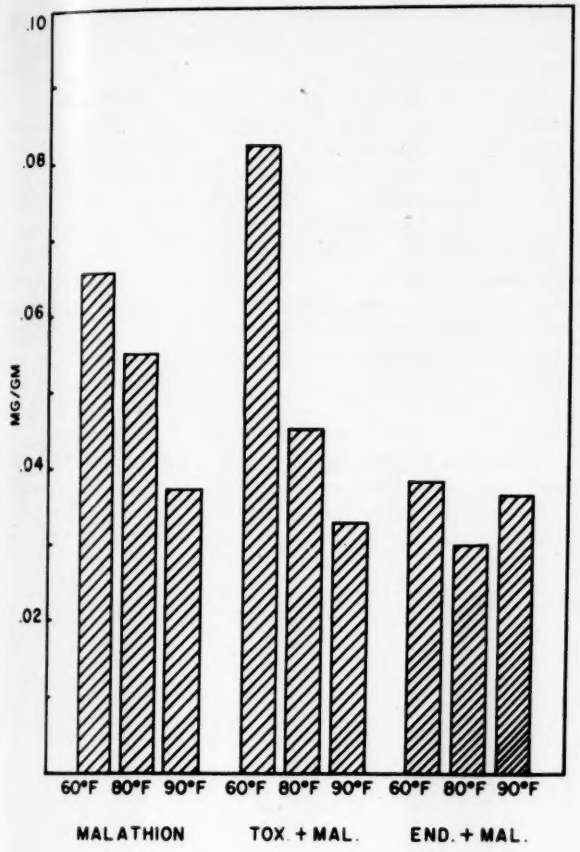


FIG. 2.—LD-50's in mg./gm. forcombined strains.

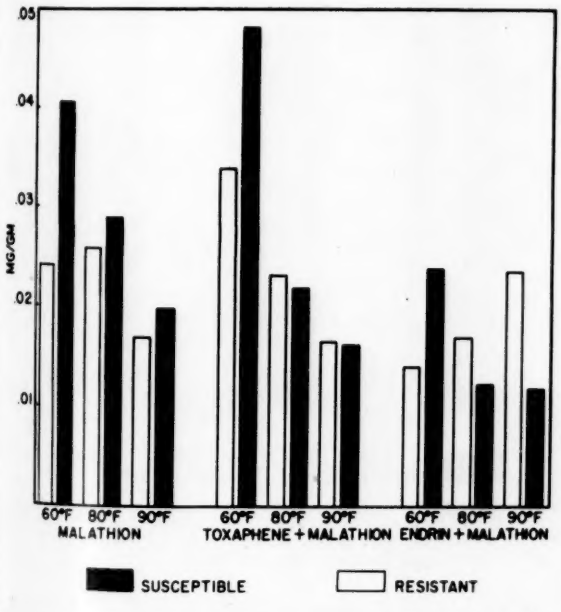


FIG. 3.—LD-50's in mg./gm. for resistant and susceptible strains at different temperature levels.

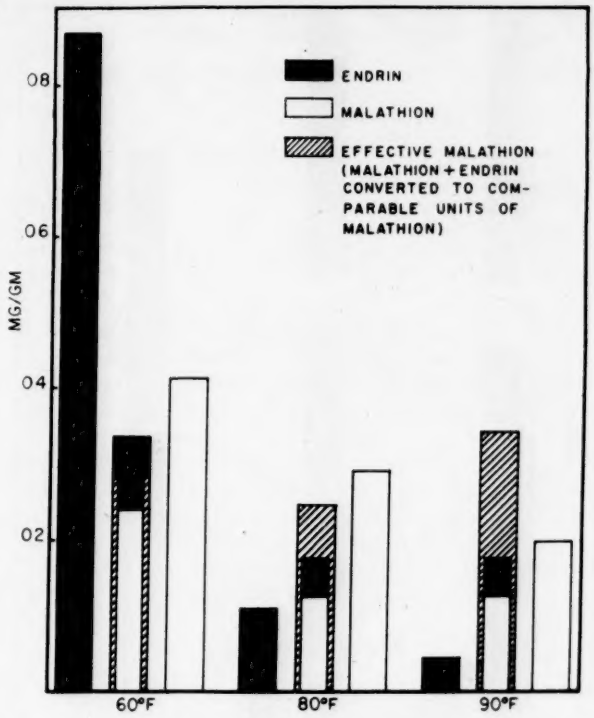


FIG. 4.—LD-50's for three insecticides (endrin, malathion, and endrin plus malathion) on the susceptible strain. Center bar for each temperature is endrin plus malathion (in the proportion of 2 to 5).

lathion combination when data for temperatures and strains were combined (fig. 1). However, LD-50's for endrin, endrin-malathion, and malathion for the reference strain at 60° and 80° F. indicate that there is synergistic action between endrin and malathion. A computation of total toxicant in the endrin-malathion mixture by adding the units of each in a 2 to 5 endrin-malathion combination required for an LD-50 would not give a true picture of the case. Rather, it is necessary to determine the ratio between the amounts of endrin and malathion required for an LD-50 when used separately. This ratio was set up as equal to the amount of endrin in the endrin-malathion combination divided by X, where X is the amount of endrin in the endrin-malathion combination converted to "effective malathion."

The result of this ratio is

$$\frac{.0874}{.0413} = \frac{.0096}{X}$$

where .0874 is the amount of endrin alone required for an LD-50, .0413 is the amount of malathion alone required for an LD-50, .0096 is the amount of endrin in the endrin-malathion combination, and X is the amount of endrin in the endrin-malathion combination converted to "effective malathion" which is 0.0045 mg./gm. This figure is then added to the amount of actual malathion in the endrin-malathion combination (.0045 mg./gm.+.0240 mg./gm.=.0285). The sum of these figures is 0.0285 mg./gm. "effective malathion" which can be compared to the LD-50 of malathion (0.0413 mg./gm.). The LD-50

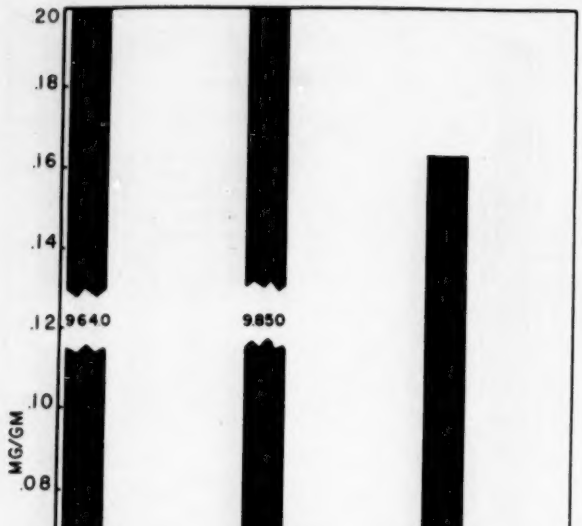


FIG. 5.—LD-50's for three insecticides (endrin, malathion and endrin plus malathion) on the resistant strain. Center bar for each temperature is endrin plus malathion (in the proportion of 2 to 5).

for the combination (0.0285 mg./gm.) when compared to 0.0413 mg./gm. the LD-50 for malathion alone, indicates that there was synergistic action between the endrin and malathion in the combination used. This is evidenced by the mortality obtained with the combination of endrin and malathion being more than the total effectiveness of each insecticide used separately. At 90° F. there was an indication of a slight antagonistic action between endrin and malathion in combination as shown in figure 4 wherein the LD-50 for endrin in combination is 0.0051 mg./gm., when compared with 0.0049 mg./gm. for endrin alone.

The same general trend of results was obtained after treatment of the resistant strain with endrin, endrin-malathion, and malathion (fig. 5) as was obtained for the reference strain. However, there was a different situation evident at 90° F. For the resistant strain considerably more malathion was required in combination with endrin than was required when used alone. When comparing the results obtained for the two strains at 90° F. it appeared that endrin was responsible for the mortality in the reference strain, and in the resistant strain mortality resulted from malathion.

The results for toxaphene, toxaphene-malathion, and malathion were quite different as far as temperature was concerned. The toxaphene in the toxaphene-malathion combination was converted to "effective malathion" by computations similar to those used in converting endrin in the endrin-malathion combination. Conversions were made for 60°, 80°, and 90° F. Comparison of LD-50's for the toxaphene-malathion combination and the malathion alone indicated that there was an antagonistic action between toxaphene and malathion at 60° F. This was true for both the resistant and reference strains. However, at 80° and 90° F. there were indications of a synergistic effect on both strains. Results for reference and resistant strains are presented in figures 6 and 7, respectively.

There was a significant interaction between insecticide and strain. Malathion and the toxaphene-malathion combination were more effective against the resistant strain. This may readily be observed when comparing the LD

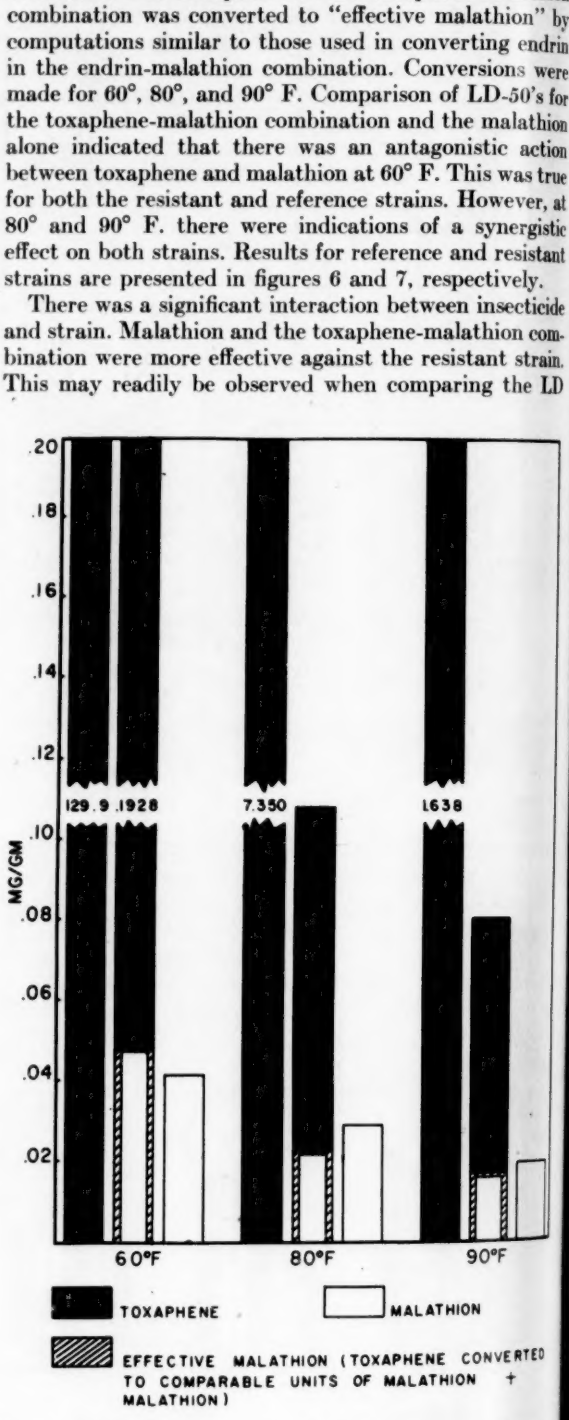


FIG. 6.—LD-50's for three insecticides (toxaphene, malathion, and toxaphene plus malathion) on the susceptible strain. Center bar is toxaphene and malathion (in the proportion of 4 to 1).

50's presented in table 5. Effectiveness of the endrin in the endrin-malathion combination probably is sufficient explanation for such an interaction. Endrin, being effective against the reference strain, could naturally increase the kill of malathion when mixed with it. Since toxaphene was relatively ineffective against the reference strain, as has been pointed out previously, it did not add to the effectiveness of the malathion when mixed with it. The fact that malathion was more effective against the resistant strain, and since toxaphene did not add to the effectiveness of malathion when combined with it, caused the combination as well as malathion alone to be more effective against the resistant strain.

The greater effectiveness of the two chlorinated hydrocarbons at the higher temperatures probably caused the LD-50's of the endrin-malathion combination and the toxaphene-malathion combination to be lower for the reference strain at higher temperatures. The LD-50 for the reference strain was greater than that of the resistant strain at the lower temperature; thus an interaction between temperature and strain is shown (fig. 3).

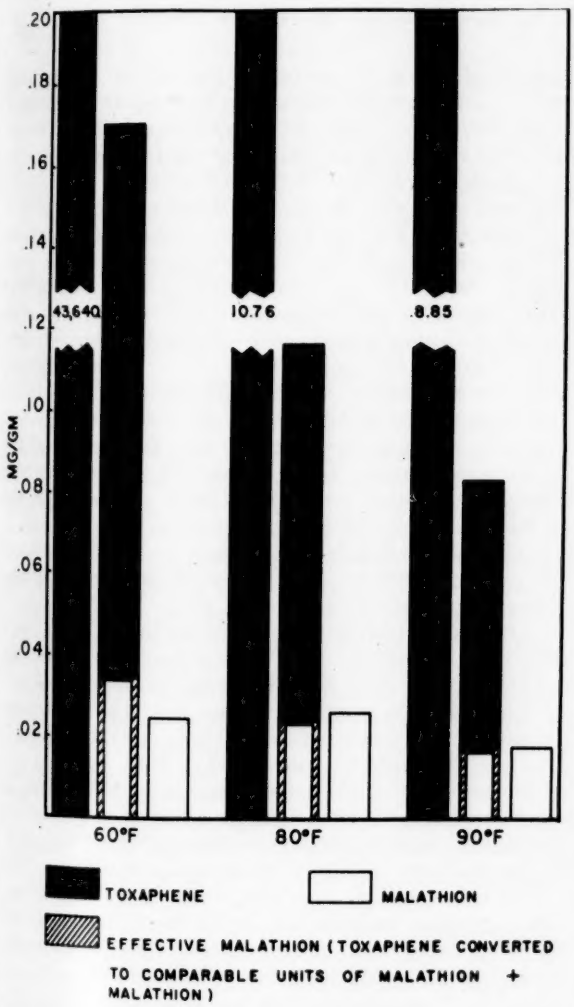


FIG. 7.—LD-50's for three insecticides (toxaphene, malathion, and toxaphene plus malathion) on the resistant strain. Center bar is toxaphene plus malathion (in the proportion of 4 to 1).

Table 5.—LD-50's of various insecticides for resistant and reference strains expressed in mg./gm. Coded by ×100.

	MALATHION	ENDRIN+ MALATHION	TOXAPHENE+ MALATHION	TOTAL
Susceptible	9.02	4.91	8.57	22.50
Resistant	6.73	5.53	7.40	19.66
Difference	2.29	−.62	1.17	2.84

The endrin-malathion combination was much more efficient at 60° and 80° F. than the other two treatments. This increased efficiency caused a significant interaction between temperature and insecticide (fig. 2). Figure 3 also shows this interaction.

Both strains show positive temperature coefficients for all insecticides with the exception of the endrin-malathion on the resistant strain. For this combination the LD-50 of the resistant strain increased as the temperature increased from 60° to 90° F.

REFERENCES CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Jour. Econ. Ent. **18**: 265.

Blum, Murray S., and C. W. Kearns. 1956. Temperature and the action of pyrethrum in the American cockroach. Jour. Econ. Ent. **49**: 862–5.

Brown, A. W. A. 1951. The effect of temperature on mortality. Insect Control by Chemicals. New York: John Wiley and Sons, Inc. London: Chapman and Hall Limited. pp. 237–43.

Dustan, G. G. 1947. Effect of temperature on toxicity of DDT. Canadian Ent. **LXXIX**: 1.

Eagleson, C. 1942. The effect of temperature on the recovery of houseflies. Soap and Sanitary Chem. **18**: 115, 141 June, 1942.

Ellisor, L. O., and C. R. Blair. 1940. Temperature and toxicity of stomach poisons. Jour. Econ. Ent. **33**: 760–2.

Fan, H. Y., T. H. Cheng, and A. G. Richards. 1948. Temperature coefficient of penetration of DDT. Physiol. Zool. **21**: 48–59.

Finney, D. J. 1952. Computation for the fitting of a probit regression equation. Probit Analysis. New York: The Syndics of the Cambridge University Press.

Guthrie, F. E. 1950. Effect of temperature on toxicity of certain organic insecticides. Jour. Econ. Ent. **43**: 559–60.

Harries, F. H., J. J. DeCoursey, and R. N. Hofmaster. 1945. Some factors affecting the insecticidal action of pyrethrin extract on the beet leafhopper. Jour. Agr. Res. **71**: 553–65.

Hoffman, R. A., and A. W. Lindquist. 1949. Effect of temperature on knockdown and mortality of house flies exposed to residues of several chlorinated hydrocarbon insecticides. Jour. Econ. Ent. **42**: 891–3.

Hurst, H. 1949. Reversible action of DDT. Nature. **163**: 286–7.

Lofgren, C. S., and L. K. Cutkomp. 1956. Toxicity of DDT to the American cockroach when lipid content and temperature are varied. Jour. Econ. Ent. **49**: 167–71.

McIntosh, A. H. 1954. Temperature coefficients of insects killed by volatile solid insecticides. Bull. Ent. Res. **45**: 137–9.

Menn, J. J., E. Benjamin, and W. M. Hoskins. 1957. The

effects of temperature and stage of life cycle upon the toxicity and metabolism of DDT in the house fly. Jour. Econ. Ent. **50**: 67–74.

Munson, Sam. C., George M. Padilla, and Max S. Weissmann. 1954. Insect lipids and insecticidal action. Jour. Econ. Ent. **47**: 578–87.

Potter, C., and E. M. Gillham. 1946. Temperature and toxicity of insecticides: **Tribolium.** Ann. Appl. Biol. **33**: 142–59.

Pradham, A. 1949. Studies on the toxicity of insecticide films. Bull. Ent. Res. **40**: 239–65.

Rai, Lallan, Saad E. D. Afifi, H. C. Fryer, and C. C. Roan. 1956. The effects of different temperatures and piperonyl butoxide on the action of malathion on susceptible and DDT resistant strains of house flies. Jour. Econ. Ent. **49**: 307–9.

Rainwater, C. F., and J. C. Gaines. 1951. Seasonal decline in the effectiveness of certain insecticides against boll weevils. Jour. Econ. Ent. **44**: 971–4.

Reiser, Raymond, Danville S. Chadbourne, Kenneth A. Kuiken, C. F. Rainwater, and E. E. Ivy. 1953. Variations in lipid content of the boll weevil and seasonal variation in its resistance to insecticides. Jour. Econ. Ent. **46**: 337–40.

Vinson, E. B., and C. W. Kearns. 1952. Temperature and the action of DDT on the American roach. Jour. Econ. Ent. **45**: 484–96.

Woodruff, Nancy. 1950. Interactions between temperature and toxicity of four insecticides applied by injection. Jour. Econ. Ent. **43**: 663–9

Field Tests with Thimet and Bayer 19639 for Cotton-Insect Control[1]

A. R. HOPKINS, R. E. FYE, and R. L. WALKER, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*[2]

ABSTRACT

Experiments were conducted to determine the effectiveness of seed treatments of Thimet and Bayer 19639, in-furrow applications of granulated Thimet made at time of planting, and side dressing applications of granulated Thimet for the control of cotton insects and the effect on the cotton plant itself. The treatments tested afforded virtually no control of the boll weevil but gave good to excellent aphid control throughout the complete growing period of the plant. There was no evidence that Thimet was taken up or translocated more readily in soils with a lower level of phosphorus. Seed treatments of Thimet and Bayer 19639 adversely affected stands. In-furrow treatments of Thimet did not affect stands. None of the treatments affected plant height. Only one treatment, the Thimet 2-pound in-furrow treatment, adversely affected fruiting. The treatments tested had no adverse effect on grade and staple or on the germination of seed produced with these treatments. Likewise, such properties as fiber strength, maturity, fineness, boll size, seed index, lint index, and per cent of lint were not affected by any of the treatments.

Experiments conducted in 1955 in South Carolina and at various locations in the Cotton Belt demonstrated the effectiveness of the systemic insecticide Thimet for the control of thrips on seedling cotton. Another new systemic, Bayer 19639, in laboratory and limited small-plot field tests also appeared promising.

In a further study of these insecticides for the control of early-season cotton insects, particularly the boll weevil, *Anthonomus grandis* Boh., field experiments were conducted at Florence in 1956. Seed and furrow treatments at the time of planting and side dressings were tested in two small-plot experiments conducted in the same field. A randomized block design was used with each treatment, which was replicated six times. The plots were three rows wide and of sufficient length to be 1/200 acre in size. Each plot was separated from the next plot by a 2-foot fallow.

EXPERIMENT 1.—Seed treatments of Thimet and Bayer 19639 and furrow treatments of granulated Thimet made at the time of planting were tested. Coker 100 wilt cotton seed from the same source was used for all plots. Eight pounds of carbon powder containing 44% of Thimet and 2% of methylcellulose was used in treating 100 pounds of seed. The Bayer 19639, 50% impregnated on activated charcoal with methylcellulose, was used at three dosages —3, 6, and 9 pounds per 100 pounds of seed.

The treated seed was planted at rates to give 1 pound of Thimet and 1, 2, and 3 pounds of Bayer 19639 and the Thimet granules were applied at 1 and 2 pounds of toxicant per acre. The seed was planted by hand on April 19 at the rate of 33 pounds per acre.

The following records were obtained: Stand counts, thrips and aphid populations, plant height, fruiting (squares and blooms), boll weevil infestation, boll weevil emergence from squares, and yield. One field-cage test was also conducted to determine the effectiveness of the treatments against overwintering boll weevils. Records were also obtained on seed and fiber properties of the cotton produced with these treatments.

Germination was delayed by adverse weather at time of planting; moisture was sufficient but temperatures were low.

The results of this experiment are shown in table 1. The seed treatments resulted in a significant reduction of stands, but the in-furrow Thimet treatments did not.

An evaluation of the effectiveness of the treatments against thrips was not possible owing to the extremely low populations in the experimental field.

Early-season aphid populations were light, but all treatments gave highly significant reductions below the untreated check. An aphid count was made on August 10 following four applications of calcium arsenate since July 20 at 15 pounds per acre to build up populations of this insect (*Aphis gossypii* Glov.). The count showed 99.9 aphids per leaf in the untreated check compared with less than one in the plots treated with Bayer 19639 at 2 and 3

[1] Accepted for publication September 3, 1957. Presented at the meeting of the Cotton States Branch of the Entomological Society of America at Birmingham, Ala., February 4–6, 1957.

[2] In cooperation with the South Carolina Agricultural Experiment Station.

Table 1.—Effectiveness of seed treatments with Thimet and Bayer 19639 and furrow treatments with Thimet against Cotton insects. Florence, S. C. 1956.

Date of Record	Untreated Check	Seed Treatments[a]				Furrow Treatments with Thimet Granules[a]		M.S.D.	
		Thimet 1	Bayer 19639 1	Bayer 19639 2	Bayer 19639 3	1	2	5%	1%
Stand count (number of plants in center row, 19.4 feet long)									
May 8	201.1	182.8	179.3	170.3	173.1	192.8	189.3	10.9	14.7
15	189.0	170.0	175.3	163.8	163.0	177.8	179.8	12.1	16.3
Aphid infestation (number per 20 plants)									
May 10	34.1	0	0.3	0.1	0.1	0.3	0.5	21.7	29.2
17	15.0	0.1	0	0.1	0.3	0.1	0	3.7	5.0
24	6.0	1.0	0.5	0	0	0.1	0.5	1.3	1.7
June 1	2.0	0.5	0.1	0	0.5	0	0	1.0	1.3
Aphid infestation (number per leaf following calcium arsenate applications)									
Aug. 10	99.0	43.9	4.8	0.3	0.3	26.1	18.0	27.2	36.7
Plant height (inches)									
May 28	3.2	3.6	3.3	3.8	3.7	3.3	3.3	[b]	
June 13	8.1	8.5	8.5	9.2	8.8	8.1	7.6	[b]	
Fruiting forms (number per plant)									
June 14	0.8	0.4	0.5	0.8	0.6	0.4	0.1	0.4	0.6
20	2.4	1.8	2.1	2.5	2.3	1.9	0.9	[b]	
27	5.4	4.9	5.4	5.7	6.0	4.9	3.9	[b]	
Boll weevil infestation (per cent of punctured squares)									
June 25	0.6	1.5	1.8	2.4	1.9	2.2	0.7	[b]	
July 17	6.5	4.8	5.3	7.5	4.1	8.6	6.8	[b]	
Bloom count (number in entire plot, 1/200 acre)									
July 9	59.8	39.8	50.0	60.0	46.0	41.3	29.8	16.2	21.8
17	69.1	66.5	67.6	68.8	65.5	61.3	61.5	[b]	
Field-cage test (number of dead boll weevils per cage)									
May 15	4.6	8.0	4.0	5.6	8.3	8.6	9.7	2.9	4.1
Final stand count (number of plants per acre)									
Aug. 15	25,800	21,860	22,600	23,000	23,240	22,560	21,420	[b]	
Yield (pounds of seed cotton per acre)									
Aug. 27	871	550	610	800	661	577	406	203	273
Oct. 8	1,350	1,790	1,797	1,757	1,813	1,670	1,887	265	356
Total	2,221	2,340	2,407	2,557	2,474	2,247	2,293	223	300
Gain over check	—	119	186	336	253	26	72		

[a] All dosages in pounds per acre.
[b] Not significant according to the *F* test.

pounds per acre. All treatments afforded some aphid control approximately 4 months after planting. The Bayer 19639 treatments resulted in a highly significant reduction below the Thimet seed treatment.

There were no significant differences between treatments with respect to plant height.

None of the treatments adversely affected fruiting except the 2-pound in-furrow Thimet, which caused some delay.

There were no significant differences between treatments with respect to the percentage of weevil-punctured squares. One field-cage test with the Thimet-seed and in-furrow treatments and the 3-pound Bayer 19639 treatment gave a significant kill of overwintering boll weevils. Records made on emergence of boll weevils from squares indicated no kill occurring within squares for any of the treatments. The data were too variable to be analyzed statistically.

On the first picking all treated plots yielded significantly less seed cotton per acre than the untreated check except the plot receiving 2 pounds of Bayer 19639, which yielded significantly more than the Thimet treatments. The second picking showed that all treatments gave significantly more seed cotton than the untreated check. When total yields were considered, the 2- and 3-pound Bayer 19639 treatments gave significantly higher yields than the untreated check.

The increased yields could not be attributed to any factors measured in this experiment. A stand count made at the time of harvest showed no significant differences in the number of plants. None of the treatments had any adverse effect on grade and staple, on the germination of seed, or on such properties as fiber strength, maturity, fineness, seed index, lint index, and per cent of lint. In the check the bolls were smaller than in the treated plots, but this was no doubt due to many uncontrolled variables.

EXPERIMENT 2.—The purpose of this experiment was to determine the effectiveness against the boll weevil of Thimet applied at excessive dosages and whether Thimet would more readily be taken up and translocated in a soil with a lower phosphorus level than in a soil with a normal level.

Side dressings were made of 2% Thimet granules at the rates of 10, 20, and 30 pounds of toxicant per acre in plots that had received fertilizers with and without phosphorus. Six hundred pounds of a 5–10–5 fertilizer were applied to one series of plots and 150 pounds of a 20–0–20 fertilizer to another, so that the same amounts of nitrogen and potash were used in all plots. The seed was planted on April 26, and the side dressings were made by

Table 2.—Effectiveness against cotton insects of excessive dosages of Thimet in soil containing soil with and without added phosphorus. Florence, S. C. 1956.

Date of Record	Phosphorus in Fertilizer[a]				No Phosphorus in Fertilizer[a]				M.S.D.	
	Check	10	20	30	Check	10	20	30	5%	1%
					Plant height (inches)					
May 25	3.0	3.0	2.9	2.7	2.7	2.6	2.9	2.8	[b]	
June 13	8.9	8.6	8.3	8.1	7.2	7.0	7.5	7.1	1.1	1.5
27	18.2	18.8	16.9	17.8	15.8	15.7	15.2	15.5	2.6	3.4
					Fruiting forms (number per plant)					
June 13	0.8	0.8	0.7	0.6	0.3	0.4	0.3	0.1	0.4	0.5
22	4.5	3.5	3.8	3.6	2.6	2.5	2.1	2.1	1.3	1.7
28	7.0	6.7	7.1	7.4	6.2	6.3	5.1	4.4	1.8	2.4
					Bloom count (number in entire plot, 1/200 acre)					
July 9	58.1	43.3	39.0	48.5	39.6	42.8	36.0	32.5	[b]	
17	60.3	47.1	44.5	52.3	51.0	51.0	44.0	46.8	[b]	
					Boll weevil infestation (per cent of punctured squares)					
June 25	3.7	4.8	0.9	1.4	1.9	3.7	3.3	2.9	[b]	
July 3	8.0	2.6	4.0	2.3	3.3	1.6	4.5	0.6	[b]	
11	2.3	2.6	2.3	1.5	4.1	2.5	1.3	1.1	[b]	
17	9.5	16.0	9.6	8.0	6.3	6.8	10.8	8.0	[b]	
					Aphid infestation (number per leaf following calcium arsenate applications)					
Aug. 10	151.6	0.2	0.3	0.1	147.7	2.2	0.2	0.1	36.1	48.4
					Final stand count (number of plants per acre)					
Oct. 15	21,660	23,040	22,360	19,440	21,960	19,960	19,840	21,350	[b]	
					Yield (pounds of seed cotton per acre)					
Oct. 8	2,085	2,385	2,232	2,367	1,939	2,379	2,240	2,044	[b]	
Gain over checks:										
Phosphorus in fertilizer	—	300	147	282	−146	294	155	−41		
No phosphorus in fertilizer	146	446	293	428	—	440	301	105		

[a] Dosages of Thimet in pounds per acre.
[b] Not significant according to the *F* test.

hand on May 25, when the cotton was in the four-true-leaf stage.

The results of this experiment, presented in table 2, show no significant differences between treatments with respect to weevil infestation, plant height, stand, fruiting, or yields.

The field-cage tests with overwintering weevils and one cage test with laboratory-reared first-generation weevils showed that these excessive rates of Thimet failed to afford any control. In experiment 1 boll weevils were killed by Thimet on seedling cotton, but in experiment 2 on cotton beginning to square and older no mortality was obtained. Records obtained on boll weevil emergence from squares showed no mortality within squares for any of the treatments, the emergence in the checks being 72% and in the treated plots 74%. The data were too variable to be analyzed statistically.

An aphid count made on August 10, following four applications of calcium arsenate since July 20 at 15 pounds per acre to build up populations of this insect, showed an average of 150 aphids per leaf in the untreated checks and less than 3 aphids per leaf for each treatment. All treatments were affording virtually complete aphid control after 2½ months.

None of the treatments had any adverse effect on grade and staple or on the germination of seed. Likewise, such properties as fiber strength, maturity, fineness, lint index, and per cent of lint were not affected by any of these treatments. Although the checks had smaller boll size and a lower seed index, it is believed that no significance should be attributed to this because of many uncontrolled variables.

NOTICE TO AUTHORS

On the inside of the front cover of the September, 1957, issue of the *Bulletin of the Entomological Society of America* (Vol. 3, No. 3) the policy of omitting the footnote designating papers for which part of the publication costs are paid (unless authors of such papers requested that it be included) was announced.

At the Annual Meeting held at Memphis, Tenn., Dec. 2–5, 1957, the Governing Board of the Society adopted a resolution requiring that papers published in the *Annals of the Entomological Society of America* and in the JOURNAL OF ECONOMIC ENTOMOLOGY heretofore carrying the footnote "Paid paper" be identified in the future by the following footnote:

"Partial cost of publication of this paper was met by . . . " (Appropriate information on agency, institution, etc., to be inserted.)

Toxicity of Pesticides to Honey Bees in Laboratory and Field Tests in Southern California, 1955–1956[1]

L. D. Anderson and E. L. Atkins, Jr.,[2] *University of California Citrus Experiment Station, Riverside*

Abstract

In laboratory tests 33 compounds were compared for relative toxicity to honey bees. Ten of these materials (mostly phosphates) were found to be more toxic than the DDT standard treatment, four were about as toxic as DDT, and all the rest (mostly phosphates) were practically nontoxic to honey bees. Sabadilla at dosages comparable to those used in citrus groves was nontoxic to bees, while dosages comparable to those used on vegetable crops were highly toxic. Studies of DDT, toxaphene, and sulfur, alone and in various combinations, showed that DDT was moderately toxic to bees, and that sulfur and toxaphene were only slightly toxic. No synergistic effects were noted in the combinations.

In field tests parathion was found to be highly toxic to honey bees, and its use is not recommended on alfalfa in bloom. Although malathion is moderately toxic to bees, it appears to be less toxic than parathion, and with proper timing of applications it has been used on blooming alfalfa without serious loss of bees. In these tests Trithion and Dipterex had no serious effects on honey bees in blooming alfalfa fields.

Comparative studies on the toxicity of various pesticides to the honey bee, *Apis mellifera* L., begun in 1950 (Anderson & Tuft 1952) and carried on since that time, were continued in the laboratory and in the field in 1955.

Laboratory Tests.—*Materials and Methods.*—These tests were made under precisely controlled conditions of temperature, humidity, and time, using the vacuum bell-jar method of dusting. This method (developed by Farrar *et al.* 1948), and the technique used in these tests have been described in previous articles by Anderson & Tuft (1952), Atkins & Anderson (1954), and Atkins *et al.* (1954).

During 1955, tests were made with 33 relatively new pesticides, many of which are still in the experimental stage of development and are not in commercial production. Most of these dusts were prepared with pyrophyllite as the diluent. With a few exceptions, all materials were tested at dosages of 100, 200 and 400 mg. Except for a few of the relatively nontoxic materials at the lower dosages, all tests were made with 9 replicates of at least 20 bees for each replicate for each material. Since tests could not be made simultaneously with all materials, 5% DDT at the 200-mg. dosage and untreated bees were included as standards in all tests. Treated bees were held in clean cages provisioned with 50% honey-water solution. They were held in a shaded greenhouse room having a constant temperature of 80° F. and a relative humidity of 65%. Observations and mortality counts were made at appropriate time intervals for 72 hours.

Results.—The materials used and the results obtained in these tests are shown in tables 1, 2, and 3. Although eight of these new materials appear to be highly toxic to honey bees, it is interesting to note that many of the new materials, such as Trithion, Dow ET-57, and Thimet, appear to be less toxic to honey bees than the standard DDT treatment. In fact, materials such as Phostex, Kelthane, and Dipterex appear to be relatively nontoxic to honey bees.

Table 1.—Comparative toxicity of pesticide dusts to honey bees at 400-mg. dosages.

Treatment No. and Compound[a]	Per Cent Toxicant	Per Cent Mortality (Hours After Treatment) 4	6	24	48	72
1. Am. Cyanamid 4124	2	69	84	99	100	—
2. Guthion	1	69	74	98	100	—
3. Dow ET-15	2	5	31	98	100	—
4. DDVP	2	16	24	81	100	—
5. Phosdrin (200-mg. dosage)	2	35	39	87	98	100
6. Am. Cyanamid 12008	2	18	24	94	99	99
7. Sevin (C-7744)[b]	2.5	15	72	96	96	96
8. Am. Cyanamid 12009	2	20	56	88	90	90
9. DDT (200-mg. dosage)	5	2	16	52	54	55
10. Trithion	5	1	9	44	47	48
11. Dow ET-57	2	4	11	40	44	44
12. Thimet	2	18	19	24	34	35
13. Karathane	1	2	3	6	9	10
14. Copper oxychloride sulfate	6[c]	1	2	5	7	8
15. Phostex (C-1137A)	2	0	1	2	4	5
16. Monohydrated copper sulfate	7[c]	0	0	1	3	4
17. Kelthane	5	1	1	3	3	3
18. Dipterex	2	1	1	2	2	3
19. Hercules AC-528	2	0	0	1	2	3
20. Chlorbenside	7.5	0	0	1	2	3
21. Methyl chlorobenzilate	4	0	1	1	2	2
22. Holcomb C-326	2	0	0	1	2	2
23. Untreated Check	—	0	0	1	2	2

[a] Definitions of most of these pesticides may be found in the lists of "Common Names of Insecticides," Jour. Econ. Ent 48(1): 112–5 (1955) and 50(2): 226–8 (1957).

These compounds were supplied by the following companies: American Cyanamid Co. (1, 6, 8, 12); Farbenfabriken Bayer AG. (2); Carbide and Carbon Chemicals Co. (7); Dow Chemical Co. (3, 11); Geigy Agricultural Chemicals (9, 21); Hercules Powder Co., Inc. (19); J. I. Holcomb Mfg. Co. (22); Montrose Chemical Co. (4, 18); Niagara Chemical Division, Food Machinery & Chemical Co. (14, 15, 16); Rohm & Haas (13, 17); Shell Oil Company (5); Stauffer Chemical Co. (10); The Upjohn Co. (20).

[b] Sevin is N-methyl-1-naphthyl carbamate.

[c] Per cent metallic copper.

In addition to the materials listed in the tables, 10 other experimental materials (eight phosphate and two non-phosphate) were tested at all three dosage levels. Of the phosphates six were nontoxic, one was moderately and one highly toxic, to the bees. One non-phosphate material was nontoxic and one was highly toxic.

Although the 2-hour mortality counts were made for all three dosage levels, the kills were so low for most of the treatments that the data are not included in the tables. The maximum mortality at 2 hours was 37% for

[1] Paper No. 980, University of California Citrus Experiment Station, Riverside. Part III of a series of papers on the toxicity of pesticides to honey bees. Presented in part at the Annual Meeting of the Pacific Branch of the Entomological Society of America held at Riverside, California, June 22–24, 1955. Accepted for publication September 3, 1957.

[2] The authors acknowledge the valuable assistance of H. T. Reynolds, P. D. Gerhardt, M. H. Frost, G. E. Printy and R. M. Hannibal in the laboratory phase of the testing.

Table 2.—Comparative toxicity of pesticide dusts to honey bees at 200-mg. dosages.

Treatment Compound[a]	Per-Cent Toxicant	Per Cent Mortality (Hours After Treatment)				
		4	6	24	48	72
Am. Cyanamid 4124	2	72	91	100	—	—
Guthion	1	64	79	99	100	—
Dow ET-15	5	1	36	99	100	—
Phosdrin	2	35	39	87	98	100
DDVP	2	15	17	83	96	100
Dow ET-15	2	0	14	69	78	78
Sevin (C-7744)	2.5	9	43	72	72	72
Am. Cyanamid 12008	2	28	41	68	72	72
DDT	5	2	16	52	54	55
Am. Cyanamid 12009	2	8	19	41	45	46
Dow ET-57	2	4	5	18	22	24
Thimet	2	4	9	14	21	22
Trithion	5	0	1	12	17	21
Dipterex	2	0	1	1	3	6
Hercules AC-528	2	1	1	2	4	5
Untreated Check	—	0	0	1	2	2
Holcomb C-326	2	0	0	0	0	0
Sabadilla (old)	20	37	80	100	—	—
Sabadilla	20	22	48	98	100	—
Sabadilla	10	3	27	47	48	48
Sabadilla	5	0	0	1	3	3
Sabadilla	1	1	2	2	3	3

[a] "Sabadilla (old)" indicates data obtained with this material in 1953 tests. Other data on sabadilla obtained in 1955 with the same (1953) lot of mixed dust. The 10, 5, and 1% mixtures were made by diluting the 20% mix with pyrolite. For further information regarding compounds, see footnotes of table 1.

Phosdrin, 35% for Guthion, 20% for Am. Cyanamid 4124, 16% for DDVP, and less than 3% for all other treatments.

Previous laboratory tests (Atkins & Anderson 1954) have shown that 20% sabadilla dust was highly toxic to honey bees. However, field use of sabadilla in citrus orchards has not appeared to affect bees seriously. When dosages were compared it was found that 1.2 ounces of alkaloid was used per acre in the bait spray on citrus, whereas a 20% dust at 30 pounds per acre, as used for vegetables, would be 9.6 ounces of alkaloid per acre. In view of these facts, laboratory tests were made with 20, 10, 5 and 1% sabadilla dusts, and as indicated in table 2, the 1% and 5% dusts were nontoxic to honey bees. The 5% dust at 30 pounds per acre is about double the sabadilla alkaloid dosage used on citrus. It is also interesting to note that the 20% dust in this test gave results almost identical with those obtained with the 20% dust material tested in 1953. Both lots of 20% material came from the same original batch.

Table 3.—Comparative toxicity of pesticide dusts to honey bees at 100-mg. dosages.

Treatment Compound[a]	Per Cent Toxicant	Per Cent Mortality (Hours After Treatment)				
		4	6	24	48	72
Am. Cyanamid 4124	2	32	83	99	100	—
DDVP	2	11	14	91	98	100
Phosdrin	2	37	38	84	96	100
Guthion	1	58	83	98	99	99
DDT (200-mg. dosage)	5	2	16	52	54	55
Am. Cyanamid 12008	2	17	27	48	52	52
Sevin (C-7744)	2.5	2	24	29	40	41
Am. Cyanamid 12009	2	1	5	20	22	22
Dow ET-15	2	0	2	10	14	14
Thimet	2	3	6	9	12	12
Trithion	5	1	1	2	4	5
Hercules AC-528	2	1	2	2	3	3
Dipterex	2	1	1	2	3	3
Check	—	0	0	1	2	2
Holcomb C-326	2	0	0	0	0	0

[a] For further information regarding compounds, see footnotes of table 1.

In view of the large amount of combination DDT-toxaphene-sulfur dust used on various agricultural crops, it seemed advisable to study such combinations on honey bees in the laboratory. In these tests, each material was compared separately as well as in combinations. In the first test all the treatments were made at the 200-mg. dosage level, but bee mortality was high and it was difficult to show differences between treatments. In the second test, a 100-mg. dosage was used and the kill was about normal or a little low. Results of the two tests were combined for presentation in table 4. These results indicate that toxaphene and sulfur alone or in combination are relatively nontoxic, while DDT alone is moderately toxic, to honey bees. The addition of sulfur or toxaphene or both to DDT does not appreciably affect the results.

FIELD TESTS.—Because of the increasing importance to beekeepers of the residual effect of phosphate materials on honey bees in the field, three field tests were run, two in 1955 and a third in 1956.

Test No. 1 (1955).—In this test an airplane spray application of DDT-parathion (1 lb.-6 oz./acre) was made at 8:00 A.M., June 10, to nearly mature potato plants in a field near Riverside, California. Honey bees in screen cages were placed in the field about 3:00 P.M. and exposed for various lengths of time, in various locations, and by various methods. Three cages (5×5×5 inches) containing approximately 30 bees per cage were used for each exposure method. Similar exposures of caged bees were made in an open area near the laboratory, where no insecticide had been used. Exposures in the field were made as follows: (1) cages carried by man walking across the field (¼ mile) in 5 minutes; (2) cages placed under the

Table 4.—Laboratory tests on honey bees with different pesticide combinations in dust form. February, 1956.[a]

Per Cent Toxicant			Per Cent Mortality 72 Hours After Treatment
DDT	Toxaphene	Sulfur	
0	15	0	8
0	0	50	6
0	15	50	6
5	0	0	55[b]
5	0	50	35
5	5	0	61
5	5	50	50
5	10	50	38
5	15	40	46
Untreated			2

[a] Results with 12 replicates of approximately 20 bees per replicate for each treatment.

[b] There were 21 replicates of this 5% DDT standard treatment, and results ranged from 46 to 61% mortality.

plants for 45 minutes; (3) cages under the plants for 2.5 hours; (4) cages under the plants overnight; (5) cages on a stake 3 feet over the tops of the plants for 2.5 hours; and (6) cages on a stake overnight. After exposure, all cages were returned to the laboratory and the bees were observed for 3 days.

When the cages were placed in the field the temperature was 79° F., the relative humidity 46%, and the wind 8 m.p.h. Rather dry, windy conditions existed throughout the period of the test. These weather conditions seemed to affect the bees more than the insecticide treatments did. In these tests mortality ranged from 5 to 49%; however, the bees exposed in the parathion-DDT-treated field showed less mortality than those in the untreated check cages. These tests indicate the lack of toxic fumigation effect of a parathion-DDT spray on bees placed in the field 6 to 8 hours after treatment.

Field Test No. 2 (1955).—This test was conducted July 25 to 29 in cooperation with J. E. Eckert, Apiculturist of the University of California at Davis, in three alfalfa fields (*A*, *B*, and *C*) in the Imperial Valley of southern California. These fields were several miles apart and had apiaries located at the edge of each field. Considerable alfalfa was in bloom in the area, and all colonies were in good condition, with the bees actively working the blossoms in the test and neighboring fields.

Field *A* was 80 acres in size and had been sprayed with malathion on July 21. The neighboring 80-acre field had been recently treated with DDT-toxaphene. These treatments had caused no serious loss of bees in the 200 colonies located on the south edge of this test field. Another neighboring field (¼ mile away) had been treated with demeton on July 8, malathion on July 18, and toxaphene-DDT on July 19 without seriously affecting bees in field *A* or the neighboring field, which had a large apiary at the edge of it. For test purposes 30 acres on the west side of field *A* were treated with 8 ounces of malathion in 6 gallons of water per acre by airplane at 9:30 A.M. on July 27 when many bees were actively working the alfalfa blossoms. This field had been irrigated on July 26 and was in fairly good bloom.

A unique method devised by J. E. Eckert was used to obtain bee population records in this and subsequent field tests. The bees were counted in an area 3 feet wide by 60 feet long during a 2-minute period. Both ends of a given counting area were marked in the dirt on the irrigation border ridge for convenience in returning to the same area for subsequent counts. The counter would walk slowly, taking 2 minutes to go the 60 feet between the marks and at the same time counting the number of bees observed in a strip 3 feet wide about 2 to 3 feet from the border. This was done by extending a bamboo pole (approximately 8 feet long) at a right angle to the border, holding it 12 to 18 inches above the top of the alfalfa plants, and counting the bees passed over by the distal 3 feet of the pole. This 3-foot section of the pole was marked at each end by a weighted string which hung down to the top of the plants. In field *A*, five of these areas were located at uniform intervals on each of three (¼ mile long) irrigation borders in the area to be treated and a similar number in the designated check area. This made 15 counting areas covering a total of 2,700 square feet of area for each plot. By this method records were taken of bee populations in field *A* at various intervals on the day before, the day of, and the day after treatment. A summary of these counts is given in table 5.

Table 5.—Honey bee population counts (active bees in 2600 square feet of area) in malathion dust test in alfalfa field A near Westmorland, Imperial County, 1955.

Time of Count[a]	Temperature, ° F.	No. of Bees	
		Treated area	Check area
	July 26		
10:00 A.M.	90	110[b]	117
12:30 P.M.	95	118[b]	143
3:00 P.M.	95	147[b]	206
5:00 P.M.	95	169[b]	198
	July 27		
8:00 A.M.	94	43[b]	95
12:01 P.M.	—	0	150*
3:30 P.M.	109	26	242*
6:30 P.M.	—	83	217*
	July 28		
10:00 A.M.	90	156	188
3:00 P.M.	—	225	205

[a] Starting time. It required about 30 minutes for three counters to obtain the 30 counts necessary for a given comparison between treated and untreated areas. Wind velocity about 5 m.p.h.; gusty on July 26 and relatively calm on July 27 and 28.

[b] Pretreatment counts, as treatments were not made until 9:30 A.M. on July 27.

* Differences highly significant.

In addition to field counts and colony observations, three cages (5×5×5 inches) of bees (average 20 bees/cage) were placed in the shade under the plants just prior to treatment and three immediately after treatment. All caged bees were provided with 50% honey-water solution as needed. Counts at 2, 6, 8, 10 and 24 hours showed 26, 55, 64, 70 and 74% mortalities, respectively, for the bees in the cages that were in the field during time of treatment. Bees in cages put in the field after time of treatment showed only 4% mortality the next day. Seven small cages (3¼×4¼×¾ inches) containing 25 to 35 bees per cage were placed in the field before spraying, and within 30 hours all of these bees were dead or affected. Considerable stinging occurred among affected bees.

The results of this test in field *A* indicate that malathion treatment reduced the field population of bees to zero within a few minutes after treatment, but 6 hours later bees were beginning to reappear in the treated area, and by the next morning activity was back to normal. It was observed that bees working the field at the time of treatment became affected within a few minutes and died in the field or soon after reaching the hive. Some of the affected bees returning to the hive caused a commotion at the hive entrance and may have been attacked by unaffected bees. Study of the bees in the hives indicated little if any effect on the colony; however, it should be pointed out that probably many of the bees in a given colony were working in areas other than the treated one.

Field *B* was about 25 acres in size and had 59 colonies of bees on the west edge of the field. This field was more mature and thus had less bloom and fewer bees than field *A*. In view of these facts only a few bee counts were made. Malathion spray at approximately the same dosage as used in field *A* was applied by airplane at 10:30 A.M. on July 27. Bee populations dropped sharply but were nearly back to normal by late afternoon and were normal or

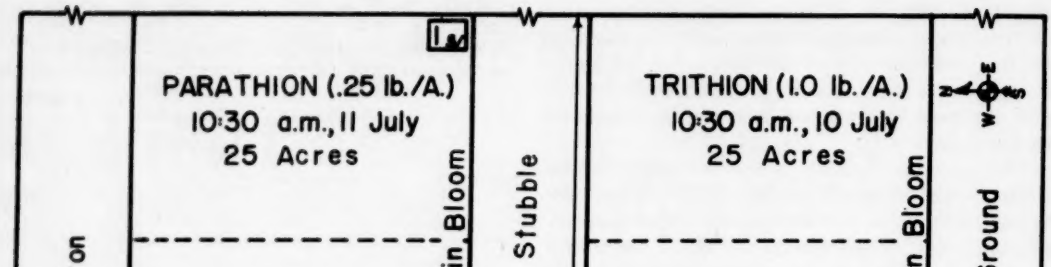


FIG. 1.—Field design of honey bee-insecticide plots (airplane-sprayed) of Test No. 3 near Westmorland, Imperial County, California, 1956. No. 1, Ashurst Apiary containing 20 colonies; No. 2, Ashurst Apiary, 28 colonies; No. 3, Beatty Apiary, more than 50 colonies; and No. 4, Deen Apiary, more than 50 colonies.

above the next morning. No untreated area was left in this field. Examination of the colonies before treatment and for 1 day after treatment indicated they were in excellent condition with no apparent ill effects as a result of the treatment. Here again part of the field force may have been working in other fields about a mile away.

Field *C*, about 25 acres in size, was very weedy and had little bloom. Twelve excellent colonies (4 in each of 3 locations) had recently been moved into this field. The field was sprayed at 9:00 A.M. on July 27 by an airplane flying directly over the bee colonies and applying parathion at the rate of 4 ounces per acre. The fronts of the hives were wet with the spray. Three cages (5×5×5 inches) containing a total of 72 bees placed in the field at time of treatment had all the bees knocked down within 5 minutes and all dead within 6 hours. One cage of 28 bees put in the field 20 minutes after the treatment had no bees down in 1 hour but 80% dead in 6 hours, 82% in 8 hours, and 96% in 24 hours (1 bee survived). Two cages (total of 52 bees) placed in the field 2 hours after treatment had 13 bees down in 3 hours, 63% dead in 7 hours and 73% dead in 24 hours. Few bees were working this particular field, and population counts were made only once before and twice after treatment. These counts showed 8 bees per 180 square feet at 2:00 P.M. the day before treatment and 4 and 6 bees per 180 square feet 7 and 24 hours, respectively, after treatment. Despite lack of bloom and other variables such as weeds, lack of bees, and fewness of samples, it appears that the bees repopulated the field in about the same time as in field *A*. In this test, spraying directly on the colonies killed a few bees, but examination of the colonies revealed no serious effect. Again, many of the bees appeared to be working in neighboring untreated fields.

During the 3 days of these tests, temperatures were near 90° F. by about 8:00 A.M., climbed to maximums of 95° to 109° F. by 3:00 P.M., and were back near 90° F. by 6:00 to 7:00 P.M. Wind conditions were mild, and the relative humidity was low for the 3 days. The fields under study had just been, or were being, irrigated during the period of these tests. Under these conditions bee populations in the fields were lowest between 7:00 and 8:00 A.M.; population levels built up until about 5:30 P.M. to 6:00 P.M., and bees were leaving the fields rapidly by 7:00 P.M. (Pacific daylight time).

Field Test No. 3 (1956).—On July 9 to 12, tests were made in which the toxicities of Dipterex, Trithion, malathion, and parathion were compared on honey bees in alfalfa seed fields near Westmorland in the Imperial Valley. These tests were made in cooperation with J. E. Eckert (as were those of Field Test No. 2) and F. E. Todd, Apiculturist, U. S. Department of Agriculture, Tucson, Arizona.

The arrangment and size of plots, materials used, and location and size of apiaries are given in figure 1. Apiaries 1 and 2 were moved into the area 2 days before treatment; apiaries 3 and 4 had been in the area for some time previously. All colonies were in good condition, and the

bees appeared to be working the various alfalfa fields in the area. The test alfalfa fields had considerable seed already set, and fresh blooms were only moderately abundant. The fields were dry and were being irrigated during the test period. The bee population in these fields appeared to be good the evening before the first treatments, but the populations were not so high on the test days. This may have been a result of the high temperatures (107° to 116° F.) on the test days. The bee population dropped to a very low point during midafternoon and late afternoon each day and was practically zero on the afternoon of the second test day.

The airplane spray treatments were well applied at 5 to 6 gallons per acre under good weather conditions, under the supervision of B. L. Mowry of the VISCO Company. Treatments were made late in the morning in the hope of catching many of the field force of bees in the field. A commercial airplane spray treatment (presumably parathion) of an alfalfa field on the southwest edge of Apiary 4 about 7:00 to 8:00 A.M. on July 10 caused some killing of bees, as observed at the colonies between 8:00 and 9:00 A.M.

Bee population counts and caged-bee records were taken in the same manner as in the first test in 1955. Records were also taken on bees placed in 3-foot-cube cages put over treated and untreated plants. In addition, records were taken on the various test colonies by counting dead bees that appeared on papers spread in front of the hives, and by inspection of the interior of the hives.

The results of the field population counts are given in table 6. Although field populations were unusually low in some of the counts, these data indicate that Dipterex and Trithion had little effect on the number of bees visiting the field after treatment, that malathion may have had some effect, and that parathion apparently reduced subsequent populations for nearly 1 day.

Results of the tests with caged bees are given in table 7. It is evident that Trithion gave a moderate kill of the bees in cages in the field at the time of treatment and practically no kill of bees placed in the field after treatment (one exception, the apparently contaminated cages, as indicated in the table). Dipterex resulted in a slight kill of treated bees and practically no kill of bees placed in the field after treatment. The data from the large 3-cubic-foot cages are meager but indicate no significant kill of bees; however, the bees were not observed to visit the flowers.

The cage tests with parathion and malathion showed 100% kill of all treated bees and of all caged bees placed in the field soon after treatments, as well as of those put in up to 3:30 P.M. Those placed in the field at 5:30 P.M. showed 90% kill for parathion and practically none for malathion. This agrees with data obtained in 1955 with parathion, except that the killing time lasted a little longer; but the malathion appeared to be decidedly more toxic in 1956 than in 1955, since no kill was obtained in 1955 of bees entered in the field after treatment. This may be due to higher dosages or higher temperatures in 1956.

The numbers of dead bees were observed at intervals in front of the various colonies. Counts of dead bees were light in front of the hives on July 10 after the Dipterex and Trithion treatments, but a moderate kill was observed in front of the colonies after the malathion and parathion treatments on July 11. Some kill was observed in Apiary 4 on July 10, as indicated above, as a result of commercial treatments to the southwest of this apiary.

Table 6.—Effect of various airplane-applied insecticide sprays on populations of honey bees in four alfalfa fields near Westmorland, Imperial County, California, 1956. Field Test No. 3.

Date and Time of Count[a]	Active Bees in 20 Samples (3600 Square-Foot Area)			
	Field A		Field B	
	Dipterex[b]	Check[c]	Trithion[b]	Check[c]
July 10— 8:30 A.M.	52.4	123.4	61.2	72.8
July 10— 3:00 P.M.	8.0	13.0	5.0	10.0
July 10— 5:00 P.M.	156.0	167.0	19.0	24.0
July 11— 6:30 P.M.	71.2	47.6	7.6	8.0
	Field C		Field D	
	Malathion[d]	Check[c]	Parathion[d]	Check[c]
July 11— 9:00 A.M.	50.0	—	71.0	79.0
July 11— 3:00 P.M.	3.8	1.2	0.0	2.6
July 11— 6:00 P.M.	6.2	8.8	17.4	47.6
July 12—10:00 A.M.	3.8	15.0	5.0	26.0

[a] A pretreatment count made at 6:30 P.M., July 9, indicated 120 to 300 bees per 20 samples in the area to be treated with Dipterex, and slightly fewer bees in the other areas. The 8:30 A.M. records of July 10 and the 9:00 A.M. records of July 11 are pretreatment counts; later records are posttreatment counts.

[b] Material applied as airplane spray at 5 to 6 gallons per acre (1 pound toxicant per acre) between 10:00 and 11:00 A.M., July 10. Temperatures and humidity records were as follows: 10:30 A.M.: T. 100° F., RH 85%; 1:30 P.M.: T. 106° F., RH 40%; 3:00 P.M.: T. 112–116° F.; 5:30 P.M.: T. 103° F. Wind slight, S.E.

[c] The check areas in Fields *A* and *C* are the same area. Likewise, check areas in Fields *B* and *D* are the same.

[d] Material applied as airplane spray at 5 to 6 gallons per acre (malathion at 0.75 pound and parathion at 0.25 pound per acre) between 10:30 and 11:00 A.M., July 11. Temperature and humidity records as follows: 8:00 A.M.: T. 90° F.; 2:30 P.M.: T. 107° F., RH 76%; 4:30 P.M.: T. 106° F.; 5:30 P.M.: T. 104° F., RH 74%; 6:30 P.M.: T. 100° F., RH 76%. Wind calm until 5:30 P.M., when it suddenly increased to 10 m.p.h. and became gusty and dusty.

Examination of the interior of the hives at the close of the test on July 12 revealed little if any serious damage to the colonies by the treatments. However, again it should be pointed out that the areas treated were relatively small and that the toxic effects of these treatments might have been of importance if the entire foraging area had been treated.

CONCLUSIONS.—The following conclusions are drawn from these field tests on honey bees in blooming alfalfa:

A spray treatment of 0.25 pound of parathion per acre killed all the field force of bees in the field at treatment time and continued to kill bees in cages placed in the field up to 4 to 6 hours after treatment, but was relatively nontoxic to bees placed in the field 6 to 8 hours after treatment.

Parathion treatment of small fields, when neighboring fields are in bloom and are not treated, does not affect colony strength to any great extent; however, an area-wide treatment with parathion or treatment of a large field could be disastrous to colonies of bees working in the area.

Parathion treatment in small areas reduces field populations of bees for 1 day.

Parathion should not be used on alfalfa in bloom.

Malathion at 0.5 and 0.75 pound per acre in 1955 and

Table 7.—Mortality of caged bees exposed for various periods in cages in alfalfa fields treated with various insecticides, 1956. Field Test No. 3.

Insecticide Application	Exposure Period[a]	Per Cent Mortality of Bees			Total No. of Bees in Test[b]
		3:00 P.M.	5:00 P.M.	9:00 P.M.	
July 10	July 10	July 10	July 10	July 11	
Trithion, applied at 10:30 A.M.	10:30–10:45 A.M.	16	33	60	100 (3)
	11:00– 1:00 P.M.[c]	16	32	56	100 (3)
	1:30– 3:30 P.M.	0	0	0	34 (1)
	3:00 P.M. (big cage)[d]	0	0	1	100+ (1)
Dipterex, applied at 11:00 A.M.	11:00–11:45 A.M.	8	10	24	67 (2)
	11:30– 1:30 P.M.	8	8	8	100 (3)
	2:00– 4:00 P.M.	0	3	3	34 (1)
	3:00 P.M. (big cage)[d]	0	0	17	100+ (1)
Untreated check		0	0	0	100 (3)
July 11	July 11	July 11	July 11	July 12	
Parathion, applied at 10:30 A.M.	10:30–10:45 A.M.	100	—	—	107 (3)
	11:00– 1:00 P.M.	100	—	—	135 (3)
	1:30– 3:30 P.M.	—	8	100	96 (3)
	5:30– 6:30 P.M.	—	0	90	117 (2)
Malathion, applied at 11:00 A.M.	11:00–11:15 A.M.	100	—	—	109 (3)
	11:30– 1:00 P.M.	100	—	—	128 (3)
	1:30– 3:30 P.M.	—	18	100	119 (3)
	5:30– 6:30 P.M.	—	0	2	100 (2)
Untreated Check		0	0	0	123 (3)

[a] The caged bees in the first period for each insecticide treatment were exposed to the spray treatment. All other caged bees were put in the field as indicated, after treatment. Cages were exposed except for a light covering of green hay on top of the cages.

[b] Figure in parenthesis indicates number of 5-×5-×5-inch screen cages used in test.

[c] The bees in this test were accidentally contaminated by the last spray pass made at the edge of the field where cages were standing; these counts are therefore similar to those obtained for the first exposure period.

[d] Large 3-foot-cube cage tests; cages were left in field until counts were made the next day.

1956, respectively, affected or killed almost all the bees in the field at the time of treatment.

Malathion did not kill any of the caged bees put in the field immediately after treatment in 1955 but did kill caged bees for 4 to 6 hours after treatment in 1956 (possibly as a result of higher dosage or higher temperatures in 1956).

Malathion appears to be less toxic, and kills bees more slowly, than parathion.

If all fields in an area are treated with malathion at about the same time, the bee loss may be heavy; however, such a condition has not been observed in Southern California to date, although considerable acreage has been treated with malathion.

Malathion-treated fields become repopulated with bees in appreciable numbers within 8 hours after treatment and populations are usually back to normal or higher in 1 day.

Dipterex at 1 pound per acre and Trithion at 1 pound per acre had little if any effect on field populations of bees.

Trithion gave a moderate kill of bees in cages in the field at the time of treatment but gave practically no kill of bees placed in the field immediately after treatment.

Dipterex resulted in a slight kill of treated caged bees but no kill of caged bees placed in the field after treatment.

Dipterex and Trithion had little if any effect on colonies of bees neighboring the treated areas.

Trithion and Dipterex are phosphate materials which apparently can be used on blooming alfalfa without seriously affecting populations of honey bees.

References Cited

Anderson, L. D., and T. O. Tuft. 1952. Toxicity of several new insecticides to honeybees. Jour. Econ. Ent. **45**(3): 466–9.

Atkins, E. L., Jr., and L. D. Anderson. 1954. Toxicity of pesticide dusts to honeybees. Jour. Econ. Ent. **47**(6): 969–72.

Atkins, E. L., Jr., L. D. Anderson, and T. O. Tuft. 1954. Equipment and technique used in laboratory evaluation of pesticide dusts in toxicological studies with honeybees. Jour. Econ. Ent. **47**(6): 965–9.

Farrar, M. D., W. C. O'Kane, and Howard W. Smith. 1948. Vacuum dusting of insects and plants. Jour. Econ. Ent. **41**(4): 647–8.

NOTICE TO AUTHORS

Authors are urged to consult the Publication Policies and Manuscript Rules and Suggestions (Vol. 49(4): 575–7) before preparing manuscripts intended for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY.

SCIENTIFIC NOTES

An Automation System for Insectaries

IRVIN LEVIN, HEINZ W. KUGLER, and HERBERT C. BARNETT,
Walter Reed Army Institute of Research, Washington, D. C.

Automation in insectaries has become increasingly important as the number of electrically operated devices for controlling ambient conditions has increased. The coordination of numerous pieces of electrical equipment which govern or affect temperature, humidity, lighting, air movement and other environmental conditions into a single integrated schedule frequently makes automation a virtual necessity.

At the Walter Reed Army Institute of Research where several exotic species of mosquitoes are being maintained for virus transmission studies, an automatic system for operating various types of electrically driven insectary equipment in an integrated 24-hour schedule has been designed and in operation for several months. Some of these mosquito species such as *Culex tritaeniorhynchus* from Japan and *Culex gelidus* from Malaya not only have exacting temperature and humidity requirements, but appear to be incapable or unwilling to mate except under very well defined lighting conditions. Rearing and maintenance of such species necessitates complex scheduling of air conditioners, humidifiers, fans, and both incandescent and fluorescent types of lamps. The principal means of controlling the scheduling and operation of these various types of equipment is the automatic variable 24-hour program timer here described. This device, in addition to turning equipment on and off after varying intervals of time, also dims and brightens lights to the lower and upper limits of their capacities on regularly scheduled bases. During such periods of simulated dawn and sunset most of the electrically operated equipment is scheduled to be inoperative, so that there will be minimal equipment noise during these mating periods.

The variable 24-hour program timer is essentially a round plate of aluminum or dural 8 inches in diameter provided with four separate "tracks." Each track consists of 120 holes, each 3/32 inch in diameter. The tracks are 11/32 inch apart (fig. 1). The plate is turned by an electric clock mechanism having a friction clutch hand set and a speed of 1 revolution in 24 hours (Synchron timing machine, Hansen Manufacturing Company, Princeton, Indiana). Rivets with flat heads are merely inserted into those track holes required to give the desired program. One rivet is equivalent to 12 minutes. Since the holes of the innermost track are spaced closer than those of the outer track, sides of the rivet heads for the innermost track must be milled so that the rivet heads fit closely. The four track-microswitches (all microswitches are Minneapolis-Honeywell No. BZ-RW204), instead of being placed in one radial line, have been mounted for sim

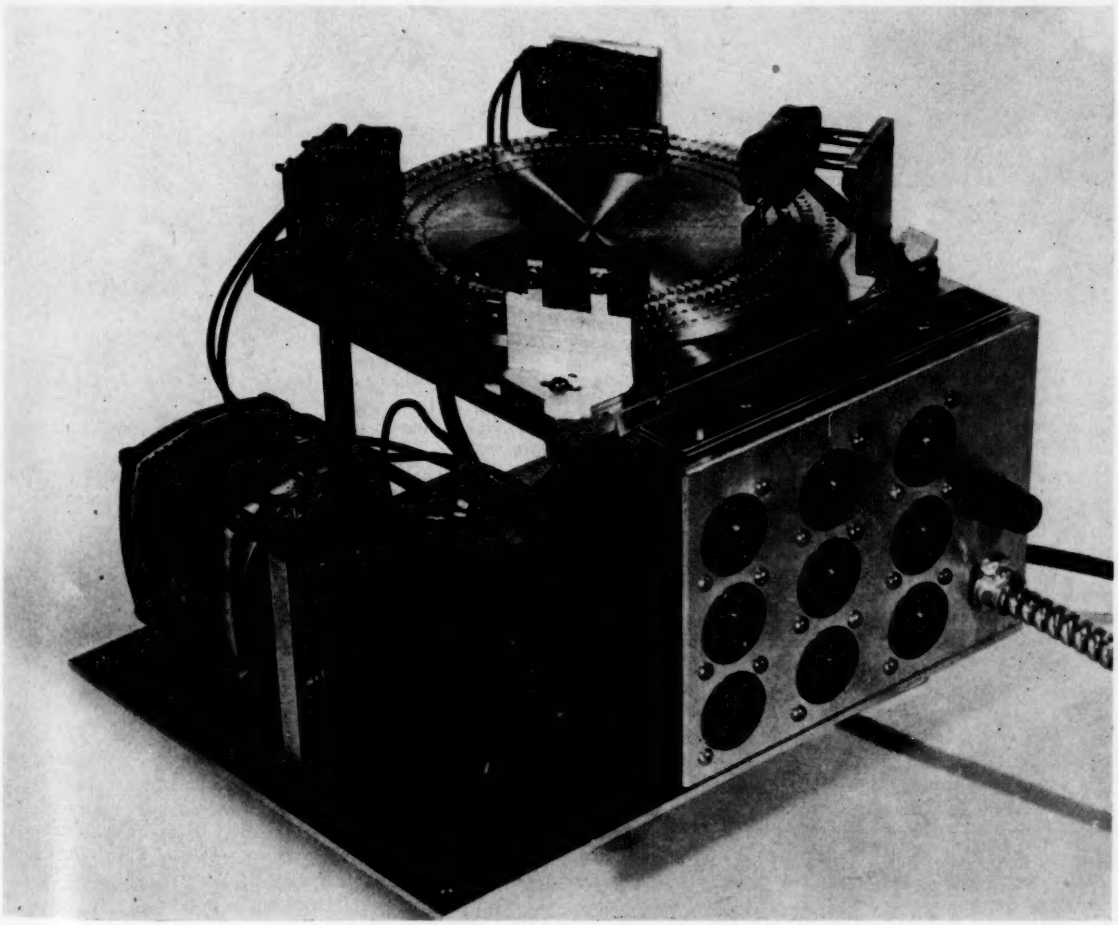


FIG. 1.—Assembled apparatus without plastic cover.

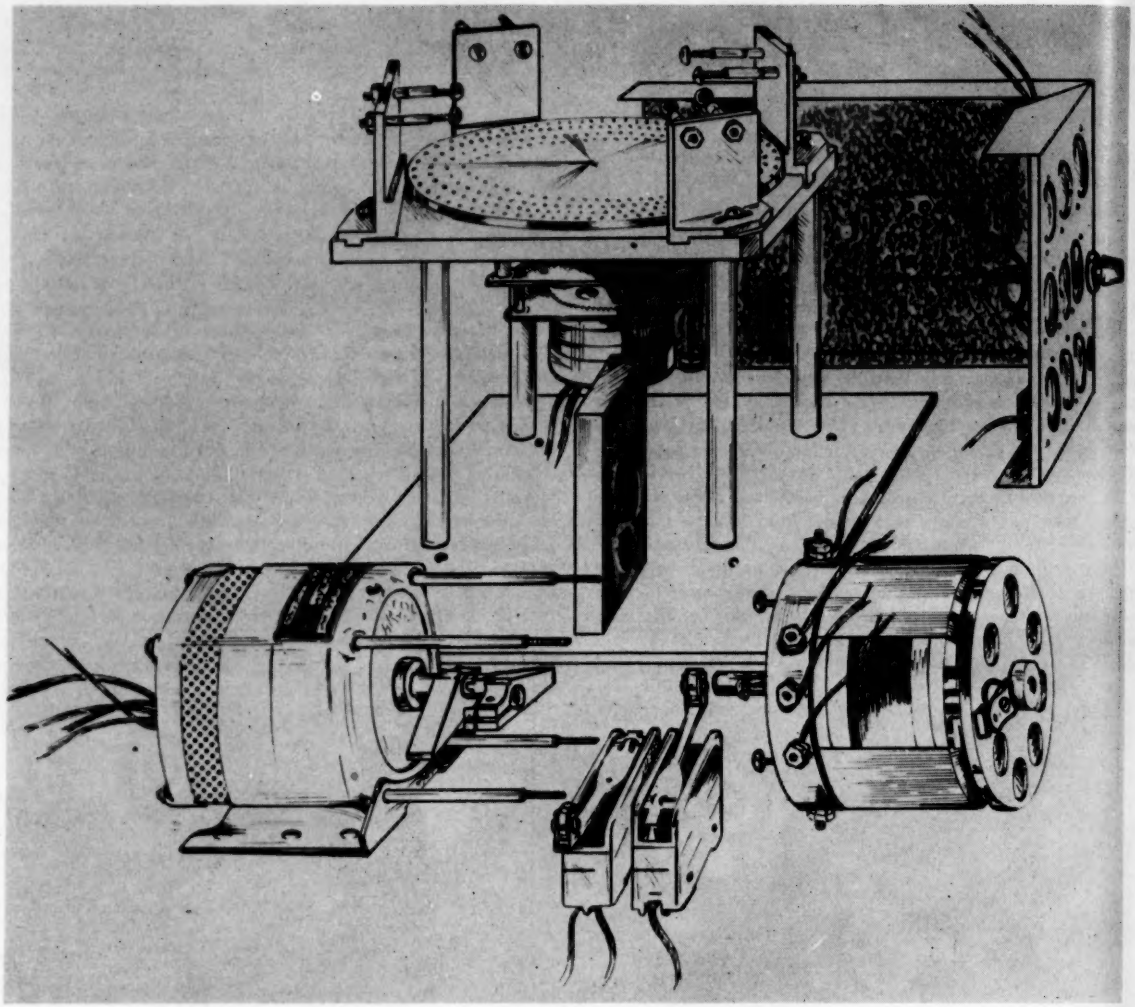


FIG. 2.—Exploded view of apparatus.

plicity at the four corners of the square plate mount, as shown in figure 2. The chassis of the apparatus is made of 1/16 inch thick aluminum sheet.

Dawn and sunset are simulated in our system by a variable transformer (Powerstat Type 20, The Superior Electric Company, Bristol, Connecticut) driven by a reversible motor (Type PYAZ, Serial No. 3–7, 0.0076 RPM no load, 0.0046 RPM full load, 100 lb. in. stall and full load torques, 115 volts, 60 cycle, 12 watts; Barber-Colman Company, Rockford, Illinois) which is controlled by the microswitch on Track 1 as shown in figure 3.

Figure 3 illustrates the electrical circuit of the apparatus. N. C. and N. O. at the microswitches represent normally closed and normally open contacts respectively. When wiring Track 4 to the wall switch, electrical code practice must be followed. The connections in our system are made directly across the original wall switch to permit turning on the overhead lights when necessary.

Figure 2 shows the two metal rotation-limiting stops mounted on the motor shaft coupling. These stops actuate their microswitches to prevent the motor from damaging the variable transformer by rotating beyond its limits. These stops can be set to shorten the duration of "dawn" and "sunset" at the expense of light intensity variation. Since the variable transformer turns through a maximum arc of about 320° and the motor has a shaft speed of approximately 1 revolution in 2 hours, the maximum duration of "dawn" and "sunset" is roughly about 1 hour and 40 minutes.

For electrical safety, the motor shaft is coupled to the variable transformer shaft by a plastic insulating coupling made of nylon. The two rotation-limiting stops are mounted on this nylon coupling.

In our system, the "day" interval is much longer than the "night" interval; therefore, to reduce rivet milling, rivets are placed only in the track holes for the "night" interval. The microswitches for Tracks 2, 3, and 4 are electrically off when they are on the rivet heads; therefore, they are connected normally closed (N. C., fig. 3).

A cover for the apparatus, made of $\frac{1}{4}$ inch thick transparent lucite sheet, protects the apparatus from dust. Each microswitch is rated to carry a maximum current of 15 amperes at 125 volts. Therefore, air-conditioners and other equipment with loads over 15 amperes per microswitch should not be controlled directly by this apparatus. For intervals less than 12 minutes per rivet, more holes are required with tracks of a larger diameter. The apparatus can easily be modified for the automatic control of other electrical equipment in the insectary.

(*Fig. 3 on next page*)

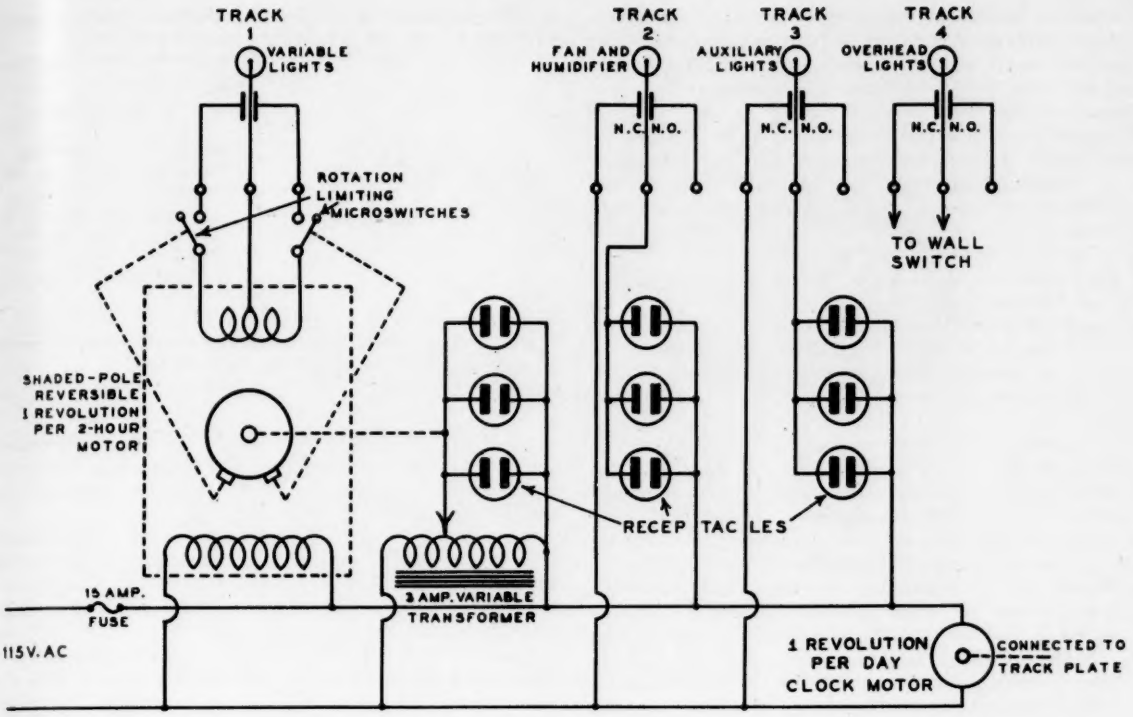


FIG. 3.—Electrical diagram of apparatus.

The Present Status of the Imported Fire Ant in Argentina[1]

KIRBY L. HAYS,[2] *A.P.I. Agricultural Experiment Station, Auburn, Alabama*

The imported fire ant, *Solenopsis saevissima richteri* Forel, is native to the subtropical and temperate climatic areas of South America. Ecologically, this is a variable area. The vegetation of the subtropical areas of Argentina is typical of that of subtropical North America. However, the general paucity of trees gives this region a plain-like appearance. The Western part of this area is semi-arid, while the Eastern part is more mesophytic.

The temperate portion of Argentina is composed largely of the area known as the "Pampas." This is a flat, almost level, treeless, grassy plain. The pastures of the "Pampas" provide the major habitat of the imported fire ant. These pastures are composed largely of native grasses; however, in some areas small scattered clovers are in evidence. The pastures provide year around grazing and no hay or silage is harvested.

The present range of the imported fire ant in Argentina extends from the province of Rio Negro, about 42° south latitude, northward to the province of Jujuy in the Andes Mountains. The Andes Mountains provide an effective barrier to a westward extension of range. The ant is also reported north of Argentina in Bolivia, Paraguay, Uruguay, and Brazil. The distribution in these countries is largely unknown; however, it is believed that the range of this ant extends into tropical South America.

Within its range the ant is found in all ecological types. Mounds may be seen in deserts, scrub savanna, and mucky river delta as well as in the better farming areas. From these observations, it appears that moisture is not a major factor in limiting the range of the ant. Altitude appears to play only a minor role in limiting its range. It has been taken from all levels of altitude from sea level to 12,000 feet. At higher altitudes it is probably temperature and not altitude that provides the barrier.

Within the range of the imported fire ant in Argentina, there is a wide variation in numbers of mounds per acre. In the fertile croplands of the province of Buenos Aires, the distribution is very spotty. The majority of the pastures have from 10 to 20 mounds per acre, whereas some have few or none. The highest infestation seen in this area was about 40 mounds per acre. Argentine specialists reported seeing infestations ranging up to 60 mounds per acre in this province. In other provinces of northern Argentina, the distribution pattern was similar. However, fewer mounds per acre were noted in the more arid sections of that country.

According to Dr. Nicolas Kusnezov, of the Instituto Miguel Lillo and one of the foremost authorities on South American ants, as many as 100 mounds per acre may be found in the province of Corrientes which lies between Uruguay and Paraguay. These observations coincide with the distributional patterns of the imported fire ant in Alabama.

In the southern areas of the range of this ant along the Rio Negro, the mounds, according to Dr. Kuznezov, became more scattered and are difficult to locate.

The food of the imported fire ant in Argentina is composed largely of insects. Argentine specialists have not noted injury to vegetation except in the building of mounds. Occasionally, ants have been observed to feed on germinating seeds; particularly those of high oil content. Most specialists consider these

[1] The information presented in this paper was gathered during a recent trip to Argentina, made at the request of the Honorable James E. Folson, Governor of Alabama. Accepted for publication September 20, 1957.

[2] The writer wishes to express his appreciation to Governor Folsom for making this trip possible, to Dr. Nicholas Kusnezov of the Institute Lillo, Tucuman, to Dr. Jose Pastrana, Head of the Division of Zoological Agriculture, Ministry of Agriculture, and to the specialists and people of Argentina who contributed to this investigation.

ants beneficial because of this insectivorous habit. Many reported seeing ants stinging, killing, and carrying numerous harmful species of insects into their mounds. One specialist estimated he had seen more than 100 different species of insects stored in the mounds of these ants.

The specialists, agricultural workers, and farmers of Argentina do not consider the imported fire ant an economic pest. Since little or no hay is harvested and farmers do not clip or mow their pastures, no damage to farm machinery was reported. No deaths of calves or other livestock resulting from fire ant attacks have been reported to Argentine authorities.

A large proportion of the agricultural produce of Argentina is harvested by hand. Persons working in citrus and apple orchards and cotton fields reported being stung only when they stood on a mound. In frequently culitvated areas, very few mounds of the imported fire ant were seen. It appears that the ants cannot tolerate cultivation or frequent disturbances of their mounds.

Three parasites of the imported fire ant have been found in Argentina. An ant, *Labauchena daguerrei* Bruch, lives on the body of the queen fire ant and steals a part of the food brought to the queen by the workers. It is also reported to eat a part of the eggs laid by the queen fire ant. In this manner it weakens the colony, but does not destroy it. Dr. Ogloblin of the Plant Quarantine Station reported a parasite in the form of the larva of a small wasp, *Orasema* sp., which parasitizes the prepupa just after the last exuvia has been shed. Parasitism by this species is very low in the field and it does not destroy the mounds that it infests. The other parasite, the larva of a small fly (Phoridae) is largely unknown. These parasites would probably be of little value in a biological control program in the United States.

Lesser Mealworms in a Brooder House[1]

WALLACE C. HARDING, JR., and THEODORE L. BISSELL,
University of Maryland, College Park

The lesser mealworm *Alphitobius diaperinus* (Panz.) (Tenebrionidae) is known as a pest of cereal products and grain that are in poor condition. It is cosmopolitan in distribution being commonly found in flour mill basements in damp or musty flour or grain (U. S. Dept. Agric., 1953).

This paper reports an unusual outbreak of lesser mealworms in the corn cob litter of a brooder house on the Eastern Shore of Maryland during which larvae were observed to bore into and apparently feed on the flesh and internal organs of moribund and dead baby chicks. Results of limited control experiments against lesser mealworm adults and larvae in corn cob litter are also presented.

LESSER MEALWORM INFESTATION IN A BROODER HOUSE.—In early September 1955, the owner of a brooder house near Centreville, Md., noticed that his corn cob litter (8 in. deep) was heavily infested with beetles and larvae which were later identified as the lesser mealworm. The owner also noted that at least a dozen of his approximately 3500 five-day-old chicks were infested with larvae of this species. The owner contacted the University of Maryland and his observations were confirmed by the writers. Subsequent observations showed that larvae attacked only chicks that were still, that is, those that were either dead or near death. In no instances were healthy moving chicks bothered. Larvae were observed to bore into the flesh and internal organs of dying and dead chicks. As many as 25 larvae were found in one chick near death.

It was concluded that the insects did not cause any deaths since only chicks that were dead or dying were infested. By the time the chicks were 10 days old 135 had died, but this was not above the expected mortality from other causes. About 80% of the 135 chicks that died were infested with lesser mealworm larvae. After the chicks reached the age of from 2 to 3 weeks no more attacks by larvae were noted, although populations in the litter remained high.

Table 1.—Effect of DDT and malathion emulsion sprays on lesser mealworm populations in corn cob litter.

SPRAY[a]	LESSER MEALWORM POPULATIONS PER QUART SAMPLE[b]	
	Before Treatment	Four Days after Treatment
	Upper 4-inch layer	
DDT, 0.5%	60.0	0.0
Malathion, 0.7%	179.5	1.0
	Lower 4-inch layer	
DDT, 0.5%	30.0	4.5
Malathion, 0.7%	57.0	11.5

[a] Rate of application was 2½ gallons to 144 square feet.
[b] Average of two samples, including both larvae and adults.

It is presumed that the lesser mealworm, being a stored-product pest, was brought in with the corn cob litter. A search of the literature did not reveal any instances where this insect infested chickens although it has been reported in corn cob litter (Gould & Moses 1951).

CONTROL EXPERIMENTS.—Since the lesser mealworm was so abundant in the brooder house, the owner planned to dispose of the litter within 4 weeks. He consented however, to the treating of two areas in the brooder house to determine if insecticidal sprays could efficiently control the insect.

Two areas, 144 square feet each, were wired off to keep chicks out. Before the sprays were applied eight quart-samples of litter (two from the top 4-inch layers and two from the bottom 4-inch layers in each of the two areas) were taken and then sifted carefully to determine the numbers of live insects present. One area was sprayed with a 0.5% DDT emulsion using 2.5 gallons to 144 square feet, and the second area was sprayed with a 0.7% malathion emulsion at the same rate. A 3-gallon compressed air sprayer was used in applying both treatments. Four days after treatment eight samples of the litter were taken as in the pretreatment sampling and the numbers of live insects determined. Table 1 gives the results of the two treatments upon lesser mealworm populations in the litter after 4 days. DDT and malathion emulsion sprays at the dosages used were effective in controlling the insects, particularly in the upper 4 inches of litter.

REFERENCES CITED

Gould, G. E., and H. E. Moses, 1951. Lesser mealworm infestation in a brooder house. Jour. Econ. Ent. **44**(2): 265.

U. S. Department of Agriculture. 1953. Stored-grain pests. Farmers' Bull. 1260. p. 46, Revised.

[1] Scientific Article No. A645, Contribution No. 2832 of the Maryland Agricultural Experiment Station, Department of Entomology. Accepted for publication October 7, 1957.

A Modified Mechanical Counter

FLOYD F. SMITH, and THOMAS J. HENNEBERRY, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

Fatigue followed the continued operation of a mechanical counter used at the Beltsville, Md., laboratory because the keys had too short radial movement. By attaching to each key a rectangular piece of clear plastic in such a way as to extend over

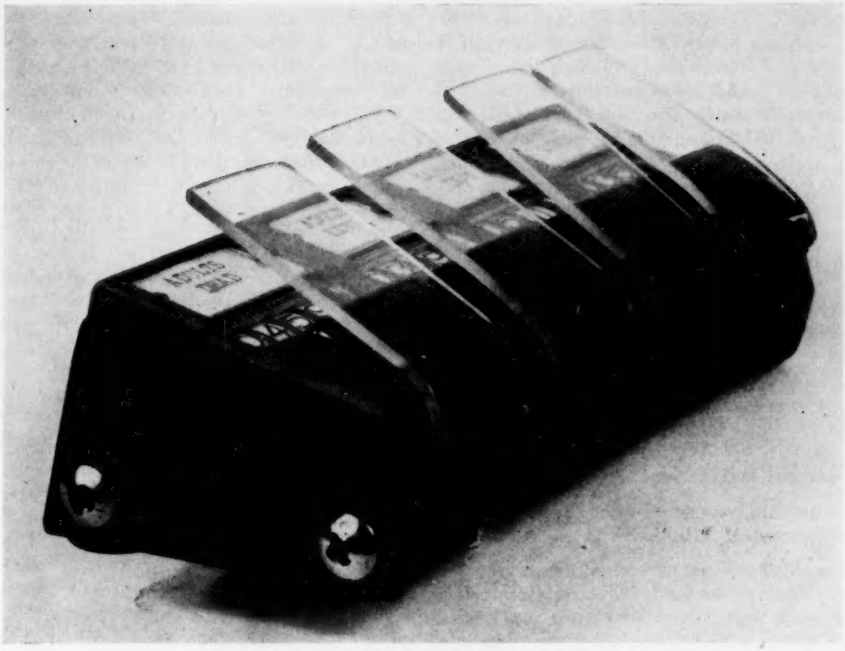


FIG. 1.—A mechanical counter with plastic extensions on keys

the counter scale, the writers found that the leverage action was increased about threefold and the finger pressure required for operation was greatly reduced. Counter recordings could be read through the plastic extensions. The plastic pieces, softened by warming, were bent to follow the curve of each key, and were fastened in place with screws.

This type of counter (fig. 1) can be made up in one or more banks by adding the desired number of units. This modification has greatly improved the ease of operating this counter over long periods.

Control of the Cabbage Looper on Cotton[1]

GEORGE P. WENE, *Texas Agricultural Experiment Station, Weslaco*

The cabbage looper, *Trichoplusia ni* (Hbn.) has recently become a troublesome pest on cotton in many areas. It feeds on the underside of the leaves and large populations will cause damage similar to that caused by the cotton leafworm, *Alabama argillacea* (Hbn.).

In the Lower Rio Grande Valley the boll weevil is the most important cotton insect problem. In 1956 two experiments were conducted for its control and data on the cabbage looper were taken incidentally while the experiments were in progress.

PROCEDURE.—The plots in both experiments were 0.1 acre in size and each treatment was replicated four times. In the first experiment, insecticides were applied with rotary hand dusters at 20 pounds per acre. The spray treatments, shown in experiment 2, of table 1, were applied with hand sprayers adjusted so that 4.5 gallons of spray were applied to an acre of cotton. Both dust and spray treatments were applied on the following dates: July 19, 22, 25, 29; July 3, 9.

Effectiveness of the various treatments against the cabbage looper was determined by selecting 10 plants at random in each plot and counting the number on 10 leaves from each of these plants. The data were taken on July 9, a few hours following the last poison application.

Table 1.—Control of the cabbage looper with insecticidal dusts and sprays applied in a seasonal program for boll weevil control.

TREATMENTS[a]	No. LOOPERS PER 100 LEAVES	PER CENT CONTROL
Experiment 1		
DUSTS:		
10% Toxaphene+2.5% methyl parathion	2.3	84
20% Toxaphene+10% DDT	2.0	86
20% Toxaphene	2.3	84
2.5% Heptachlor+2.5% methyl parathion+5% DDT	1.5	90
5% Heptachlor+5% DDT	11.8	17
4% Thiodan+5% DDT	3.3	77
5% Methyl parathion+5% DDT	0.8	94
Untreated	14.3	—
Experiment 2		
SPRAYS:		
0.25 heptachlor+1.25 malathion+1 DDT	1.8	66
0.5 heptachlor+1 DDT	5.8	0
1 toxaphene+1.25 malathion+1 DDT	0.3	94
2 toxaphene+1 DDT	1.3	75
2.5 malathion+1 DDT	0.0	100
Untreated	5.3	—

[a] Dusts applied at a total of 20 pounds per acre; sprays at pounds of active ingredient per acre of each insecticide as shown.

RESULTS.—In the first experiment a considerable amount of "ragging" by cabbage looper feeding occurred in the untreated plots and also in the plots that had been dusted with a mixture of 5% heptachlor and 5% DDT. The data show that a dust mixture of 5% methyl parathion and 5% DDT was highly effective, followed by toxaphene; toxaphene with DDT or methyl parathion; a mixture of heptachlor, methyl parathion and DDT, and Thiodan with DDT, all of which gave satisfactory control.

[1] Accepted for publication October 30, 1957.

In the spray tests the combination of heptachlor and DDT failed to control the cabbage looper. Malathion, at 2.5 pounds with 1 pound of DDT gave effective control. This agrees with Bibby (1957) in that high poundages of malathion are required for cabbage looper control. The data also show that the addition of malathion to a toxaphene-DDT mixture increased the degree of control obtained.

These data indicate that in a boll weevil control program, cabbage looper damage can be minimized by the proper selection of insecticides.

REFERENCE CITED

Bibby, F. F. 1957. Field tests of insecticidal sprays for control of cabbage looper on lettuce. Jour. Econ. Ent. 50(1): 101–2.

Boll Weevil Control with Heptachlor Granules

GEORGE P. WENE and MICHAEL SCHUSTER, *Texas Agricultural Experiment Station, Weslaco*

The use of heptachlor granules for control of early-season boll weevils has been the subject of considerable interest during 1956 and 1957. An experiment was conducted during 1957 in the Lower Rio Grande Valley in a field where boll weevils appeared every year in destructive numbers.

The experiment was conducted on three 2-acre blocks, with 24 rows of cotton in each block. The middle block was used as the untreated area, whereas the outside blocks were treated with 2.5% heptachlor granules applied at the rates shown in the table. The first and third treatments were applied with ground equipment, whereas the second was applied by airplane. Adjacent to the experimental block was a 14-acre field of cotton which was treated for boll weevil in the conventional manner by the grower. Boll weevil infestations were determined by examining 100 squares in various parts of the field and recording the punctured squares.

For the past 3 years the boll weevil infestation always developed early in the southern portion of this field, usually to a depth of 150 feet. For a month or so the infestation would be localized and the weevils would not move out of the infested area until the square infestation approached 50% as shown by the data presented in the table.

The first treatment with granulated heptachlor was made when the boll weevil emerged from hibernation or moved into the field. The percentage of square infestation remained the same in both the treated and the untreated check plots for 2 weeks after the initial treatment. This may be expected since this injury came from weevils which had moved into the field. Data taken approximately 6 weeks after the initial treatment showed more injury in the untreated plot, indicating boll weevil control. As the boll weevil infestation spread over the field the infestation was greater in the untreated plot. Counts of squares punctured by the boll weevil taken in the portion of the 14-acre field adjacent to the experimental plots showed approximately the same percentage punctured as in the untreated plot even though the field was sprayed five times for boll weevil control.

The results of this experiment indicate that applications of heptachlor granules to early localized boll weevil infestations will tend to prevent injury early in the cotton season.

Species of *Thysanus* as Primary Parasites[1]

PAUL DEBACH,[2] CHARLES E. KENNETT,[3] and ROY J. PENCE[4]

In the early days species of *Thysanus* were assumed to be primary parasites because they were commonly reared from mealybugs and scales. According to Girault (1913) "The species destroy scale insects, Coccidae, and the mealy-winged flies, Aleurodidae." This belief was based entirely on rearing records from various hosts without any careful study as to whether the emerging parasites were primary or secondary in habit, in fact Girault states that "Little or nothing is known about the habits of the Signiphorinae other than the gross food habits of their young."

Later, specialists working with parasitic Hymenoptera frequently have assumed that species of *Thysanus* reared from coccids or other hosts were hyperparasites because the only species whose life histories have been determined were found to be hyperparasitic (see references in Muesebeck *et al.* 1951). According to Clausen (1940) "they had been assumed to be primary parasites, but those upon which close observations have been made have proved to be hyperparasitic in habit. It is probable that the great majority of representatives of the family develop in this way."

The authors of this paper, working independently, have found that species of *Thysanus* are common and efficient primary parasites of the greedy scale, *Hemiberlesia rapax* (Comstock) and the latania scale, *H. lataniae* (Sign.). At least two species of *Thysanus* have been reared as primary parasites of these two scales; *T. merceti* Mal., an all black form, and *T. thoreauini* (Gir.), a brownish yellow form.[5]

BIOLOGY.—*Thysanus merceti*, a primary parasite of greedy scale, has a rather unusual life history which may represent a link between the ecto- and endoparasitic habit. Only general details have been determined thus far.

Oviposition may require a fairly lengthy period to judge from the few periods timed which ran from 14 to 24 minutes. Mature greedy scales, even those actively producing young, are preferred for oviposition. The egg is deposited free in the body cavity of the scale and will float free when the scale body is dissected in Ringer's solution. The *Thysanus merceti* egg is elongately oval

[1] Accepted for publication November 27, 1957.
[2] Department of Biological Control, University of California, Riverside.
[3] Department of Biological Control, University of California, Berkeley.
[4] Department of Entomology, University of California, Los Angeles.
[5] Determinations by H. Compere.

Table 1.—Effectiveness of 2½% heptachlor granules against an early localized boll weevil infestation.

Date of		Per Cent Boll Weevil Infestation in Various Parts of the Field					
		South		Middle		North	
Treatment	Square Count	Treated	Untreated	Treated	Untreated	Treated	Untreated
May 15 (33 lbs./A.)	May 15	8	1	0	0	2	1
	May 24	10	24	2	0	0	0
May 29 (50 lbs./A.)	May 31	50	48	2	24	6	0
June 10 (35 lbs./A.)	June 6	31	58	10	30	2	8
	June 13	28	80	3	16	5	6

and virtually exactly the same length as a mature greedy scale egg but much narrower, being four times as long as wide. When newly laid the egg has a yellow-white central mass with the remainder opaque and uncolored, except for the ends, each of which has a small clear cap. Later in development the egg becomes filled with what appear to be white fat globules, although the clear caps persist at the ends. After hatching, larvae are frequently found with the anal end associated with the host tracheae.

The development of the internal parasite larva does not stop egg production by the scale, at least up through the development of half-grown parasite larvae. After about 5 to 6 days at 80° F. when the larvae appear to be one-third to one-half grown, they migrate slowly through the host derm and then take up an external feeding position and become typical ectoparasites. Tracheal trunks and open spiracles are readily visible at this stage. The mature external larva has 14 apparent segments. Counting the head segment as number 1, open spiracles occur laterally on the third, fifth, sixth and seventh segments.

The mature larva casts meconia and pupates externally to the completely reduced scale body but under the protective scale cover. Emergence of the adult occurs after about 25 days at room temperatures.

This species appears to be uniparental. No males have been taken in our rearings.

The host-feeding habit occurs with *Thysanus merceti*. As in other parasite species exhibiting this phenomenon, host-feeding probably fulfills the adult's need for protein in order to continue prolonged egg production. The act of host-feeding may take as long as 40 minutes, including 20 minutes spent in drilling and construction of the feeding tube with the ovipositor and 20 minutes spent in sucking host fluids. The mouth of the adult parasite appears to be applied directly to the surface of the scale cover during host-feeding. The adult remains motionless during the feeding period except for slight vibrations of the antennae.

Thysanus merceti also is a common primary parasite of the latania scale, *Hemiberlesia lataniae*, on avocados. Rearing records of other species of *Thysanus* from other species of diaspine scales indicate the primary habit. It appears probable that the primary habit on diaspine scales will be found to be more common in this genus.

Marietta mexicana (How.) has been reared as a hyperparasite on *Thysanus merceti*.

ECONOMIC IMPORTANCE.—*Thysanus merceti* is frequently found to be the only parasite attacking greedy scale infesting citrus fruits, and a high degree of parasitization is often observed. Generally, commercially injurious infestations are rare. Usually only old, over-mature orange fruits show any number of greedy scales, but one general grove infestation near Santa Paula has been followed closely. This developed during 1950 in a semi-abandoned grove 2 years after the last insecticidal treatment.

Early in 1951 greedy scale was generally distributed and heavy in this grove, but by June *Thysanus merceti* and *T. thoreauini*, acting as primary parasites, were common, with *Aspidiotiphagus citrinus* (Craw.) less so. In August 1951 the major proportion of greedy scale had been parasitized. Adults of the black *T. merceti* were particularly common. A DDT-treated check tree, which inhibited parasite work, showed a much heavier infestation of greedy scale and demonstrated experimentally that parasites definitely were responsible for reducing the greedy scale population.

Early in 1952 greedy scale populations were very low in this grove with about 95% of the scale present dead. There was a slight tendency for the greedy scale population to increase during the summer of 1952, but by fall the parasites again had become common and nearly all scales were dead and/or showed *Thysanus* emergence holes. New fruit at this time was completely clean —biological control was achieved. *Thysanus* spp. were the major factors responsible for this cleanup, and based on this and other observations *Thysanus* spp. are thought to be one of the more important factors maintaining greedy scale below commercial levels on citrus and other plants.

REFERENCES CITED

Clausen, Curtis P. 1940. Entomophagous insects. 1–688. McGraw-Hill, N. Y.

Girault, A. Arsène. 1913. A systematic monograph of the Chalcidoid Hymenoptera of the subfamily Signiphorinae. Proc. U. S. Natl. Mus. 45(1977): 189–233.

Muesebeck, C. F. W., Karl V. Krombein, and Henry K. Townes. 1951. Hymenoptera of America north of Mexico—Synoptic Catalog. U. S. Dept. Agric., Agric. Monogr. 2: 1–1420. Washington.

Laboratory Study on the Effect of Thrips Infestation on the Height and Weight of Seedling Cotton[1]

B. G. HIGHTOWER, *Texas Agricultural Experiment Station, College Station*

According to Gaines (1934), *Frankliniella tritici* (Fitch), the flower thrips, and *Frankliniella fusca* (Hinds), the tobacco thrips, are the two species responsible for much of the thrips damage to seedling cotton in Central Texas. Hightower and Martin (1956) reported that *F. fusca* was the most common species during 1954 and 1955. Newsom *et al.* (1953) reported results of field experiments in Louisiana in which severe infestations of *F. fusca* reduced the leaf surface of seedling cotton plants by 50% or more and the height by approximately 20% by the time the plants were 6 weeks old.

With the above findings serving as a basis, an effort was made to determine the relative plant-damaging potential of *F. tritici* and *F. fusca* under laboratory conditions. The study reported herein consisted of a series of three laboratory experiments conducted at College Station during May, 1956, and should be considered preliminary in nature.

EXPERIMENTAL PROCEDURE.—The cotton seedlings used in these tests were grown in small clay pots filled with a 1:1 mixture of loam soil and sand. The seedlings were watered daily throughout the duration of the test. Each pot contained one plant. The tests were begun when the plants were 2 days old. Thirty plants were used in each test—10 were infested with 10 adults of *F. tritici*, 10 with an equal number of *F. fusca*, and 10 served as checks.

Specimens of *F. fusca* were taken from field-grown seedling cotton plants and *F. tritici* were taken from blooms of an evening primrose, *Oenothera speciosa* Nutt. Temperatures in the cabinets where the plants were held ranged from 80 to 87° F. and the relative humidity varied from 50 to 90%. At the end of 2 weeks, the plants were pulled up, freed of soil, weighed and measured. Measurements were taken from the crown of the plant to the base of the cotyledons.

RESULTS.—After 2 weeks the cotyledons on the plants infested with *F. fusca* showed extensive tissue damage. In several plants they were completely destroyed. Only five plants among the seedlings infested with *F. tritici* showed severe injury, although most of the cotyledons showed evidence of feeding.

Data recorded on the height and weight of the plant are summarized in table 1. Stem growth in plants infested with *F. fusca* was consistently reduced about 28% below that of the check plants, while the green weight was reduced by about 50%. Comparisons among means showed a significant difference be-

[1] Technical Contribution No. 2821, Texas Agricultural Experiment Station in cooperation with Entomology Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Accepted for publication November 25, 1957.

Table 1.—Effect of thrips infestation on the height and weight of seedling cotton plants.

	Test 1	Per Cent Reduction	Test 2	Per Cent Reduction	Test 3	Per Cent Reduction
			Mean Height in Millimeters[a]			
Check	12.3		13.8		12.9	
F. tritici	11.5	7	12.7	8	11.0*	15
F. fusca	8.8*	28	9.7*	30	9.4*	27
			Mean Weight in Grams[b]			
Check	0.77		0.63		0.63	
F. tritici	.60	22	.56	11	.33*	48
F. fusca	.33*	57	.35*	44	.23*	63

* Significant at 5% level. No significant difference between other means.
[a] Average of 10 plants.
[b] Average green weight of 10 plants.

tween the mean height of the plants infested with *F. fusca* and the mean height of both the check plants and the plants infested with *F. tritici* in tests 1 and 2. There was no significant difference between the latter two means. This was also true of the weight means in these two tests. In the third test the mean height and mean weight of the thrips-infested plants were significantly different from those of the check plants, but there was no significant difference between the plants infested with the two different species.

On the basis of these limited observations it appears that the plant damaging potential of *F. fusca* is greater than that of *F. tritici*, especially on seedling cotton.

References Cited

Gaines, J. C. 1934. A preliminary study of thrips on seedling cotton with special reference to the population, migration and injury. Jour. Econ. Ent. **27**: 740.

Hightower, B. G., and D. F. Martin. 1956. Ecological studies of thrips found on cotton in Central Texas. Jour. Econ. Ent. **49**: 423.

Newsom, L. D., J. S. Roussel, and C. E. Smith. 1953. The tobacco thrips, its seasonal history and status as a cotton pest. Louisiana Agric. Expt. Sta. Tech. Bull. **474.**

BOOK REVIEWS

(*Continued from page 58*)

ATOMIC ENERGY AND AGRICULTURE, edited by C. L. Comar with associate editors, R. F. Reitemeir, H. B. Tukey, H. Patrick, and B. F. Trum. Publication #49 of the American Association for the Advancement of Science, Washington, D. C., 1957, 450+x pp., 44 Illus., $9.50 (prepaid to AAAS members $8.25).

The 8 years between the first conference on the "Use of Radioisotopes in Agriculture" at Auburn, Alabama in 1947 and the 1955 symposium were periods of tremendous activity. The present volume is a direct result of that activity. The book is a compilation of the papers presented at the symposium on "Atomic Energy in Agriculture" held on December 27–29, 1955 at the Atlanta, Georgia meeting of the American Association for the Advancement of Science. The symposium was sponsored jointly by the Association and the Oak Ridge Institute of Nuclear Studies. Dr. Comar, formerly Chief of Biomedical Research at the Oak Ridge Institute and recently named Director of the Laboratory of Radiation Biology at the Veterinary College of Cornell University, was chairman of the symposium.

In the preface, Editor Comar states that at the symposium emphasis was upon "the critical evaluation of past accomplishments to serve as a solid basis for the future approach."

Organized into 22 chapters each by different authors, the subjects covered can be roughly grouped into four main headings. Chapters 1 through 5 relate to soil plant relationships; Chapters 6 through 12 to plant metabolism, crop improvement and protection; Chapters 13 through 16 to animal nutrition and metabolism; and Chapters 17 through 22 are concerned with the feasibility of sterilizing food by the use of ionizing radiations.

Of particular entomological interest is Chapter 12, "Radioisotopes in Entomology" by Dale W. Jenkins. Included in the 35-page Chapter are comments on the use of radioisotopes in the study of dispersal of insects, abundance and size of insect populations, the transmission of disease, insect physiology, and the mode of action of insecticides. The biosynthesis by insects of complex radioactive chemicals from simpler tagged compounds, and the dispersal by airplanes of labeled insecticides, are also covered. Included are discussions of the absorption and transmission through plants of radioactive systemic insecticides, and the synthesis by plants grown in an atmosphere of $C^{14}O_2$ of various botanical insecticides such as pyrethrum and nicotine. The section on the effects of radiation on insects covers eight pages and there are eight and one-half pages of references.

Also of direct interest to entomologists are the chapters on the radiation sterilization of food, especially Chapter 22, "Feasibility of Food Irradiation" by R. G. H. Siu. Since bacterial sterilization requires higher levels of radiation than required for killing insects, the destruction of all insect life in the food or food products would be assured. According to Dr. Siu, "Three years ago it was felt the process was a gamble. Now we are quite confident of its commercial feasibility." Chapter 22 is the only one in the book without references.

The editors have achieved, for a review-type book, a very readable volume. Considerable detail is included as well as many graphs, charts and illustrations. It is regrettable, of course, that almost 2 years have elapsed between the symposium and the publication of the book.

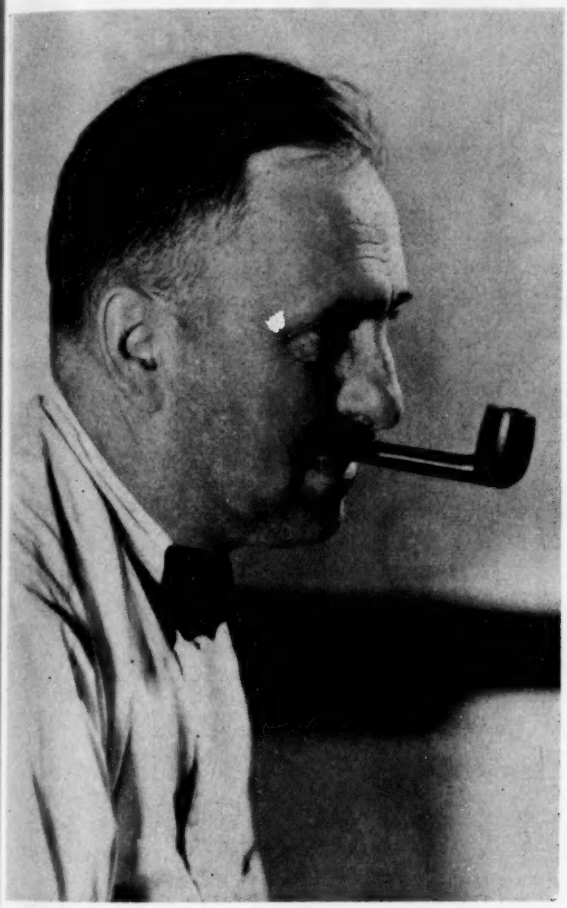
All workers in agricultural and related fields of research will find the book a useful addition to their library. Those who already use radioisotopes will refer to it repeatedly for background information. Those who have not yet used radioisotopes may well find a new approach that will aid greatly in the solution of their problem.

FRANK H. BABERS

OBITUARIES

Dwight Fletcher Barnes
1890-1957

Dwight F. Barnes was born in Hubbardston, Massachusetts, the youngest son of J. Fletcher and Anna Barnes. His brothers were Chester Gurnee Barnes, a farmer in Massachusetts, and Parker Thayer Barnes, a longtime employee of the Pennsylvania Department of Agriculture at Harrisburg under J. G. Sanders, Entomologist. When Dwight was quite young his parents moved to Marshfield, Mass. where he attended elementary schools and 1 year of high school. A schoolmate there was Mary A. M.



Baker. They were married December 23, 1916 in Brooklyn, New York. One child, Dwight Hazeltine Barnes now of Modesto, California, was born to "Dwight and Molly," as their many friends knew them, and there are three grandchildren.

From 1907 to 1910 Dwight attended Mount Hermon School, Mount Hermon, Mass. After a year of work as a carpenter he entered Massachusetts Agricultural College, Amherst, and completed his freshman year after which he put in a year of farm work. He returned to the college in the fall of 1912 as a member of the writer's class and graduated in 1916 as an entomologist. During college Dwight held several of the choicest student jobs, including janitor of the East Experiment Station and of Fernald Hall, the entomology building. His rooms in those buildings were rendezvous for his friends.

The original white pine blister rust control project, started in 1916, gave Dwight his first post-graduation job. This was followed by work as a nursery inspector for the State of Massachusetts, from which he went to the Gypsy Moth Laboratory of the U. S. Department of Agriculture at Melrose Highlands, Mass., under the strict but fair supervision of Albert F. Burgess. In November 1917 Dwight entered the U. S. Army Signal Corps as an aviation cadet. Ground Schools at M.I.T. and Cornell were followed by primary training at Lake Charles, La. He was commissioned 2nd Lieutenant in May 1918 and went overseas in September. His foreign assignments were in France and Germany.

Service at the Gypsy Moth Laboratory was resumed in August 1919 and continued until 1930 with the exception of an interval in commercial insecticide work, January 1920 to May 1922. In the latter month Dwight was put in charge of the first experimental airplane dusting undertaken by the U. S. Department of Agriculture. The following year he tried out a dirigible balloon for the same purpose. Both operations were in New Hampshire. Later, in 1926 and 1927, airplane dusting experiments were resumed, this time on Cape Cod.

Asthma became an increasing burden to Dwight and when an opportunity came to move to Fresno, California, on dried fruit insects research, he accepted. For about 8 years the change of climate practically eliminated trouble from asthma but it gradually returned to plague him for the rest of his life. A cerebral hemorrhage, about 8 years ago, was survived with complete recovery but in the fall of 1957 a series of strokes ended in his death on October 11 at the Veterans Administration Hospital, Fresno.

Outstanding characteristics of Dwight Barnes' professional life were unsparing suspicion of his research results; willingness, as he put it, to "bitter end" a project if necessary; diligence, and ability to win the confidence, respect, and friendship of growers and packers in the dried fruit industry. He became an authority on fig and date insects and on such grape and raisin insects as the grape leaf folder and raisin moth. One of his best pieces of work resulted from research on the day and night flight habits of the moths and beetles which attack dried fruits. Dwight's publications, as author or co-author, numbered about 100, several of them on forest insects and the remainder on insect pests of fruits and dried fruits.

In 1952 the California Fig Institute gave Dwight an Award of Merit and in 1957 he received from the U. S. Department of Agriculture Agricultural Marketing Service a Certificate of Merit and a cash award.

In his years of early manhood Dwight Barnes was fond of fishing, snowshoeing, and camping. During the latter part of his residence in Fresno he and Molly became much interested in raising prize-winning cocker spaniels. They had several of the best of the breed, gathered many prizes, attended numerous shows, and, as always on their journey through life, made new friends.

PEREZ SIMMONS

Mathis Charles Tanner
1891-1957

Mathis Charles Tanner, was born December 4, 1891, at Ogden, Utah. He died at Ogden, Utah on August 1, 1957. He is survived by his wife Mina Jarman Tanner, a son Allen J. Tanner and a daughter Edith Louise Tanner.

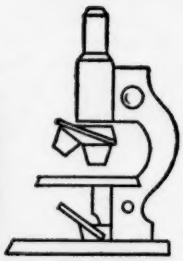
Mr. Tanner received his B.S. Degree from Brigham Young University in 1921 and his M.S. Degree from the University of Utah in 1942. His master's thesis dealt with the Plecoptera of Utah. His interest in the biological sciences and insect studies persisted throughout his life. Since 1937 he has done much interesting work with color photography. At his death he was a member of the Society of Sigma Xi, the Entomological Society of America, American Association for the Advancement of Science, Limnological and Oceanographic Society of America, and the Utah Academy of Science, Arts, and Letters. He was a veteran of World War I, where he served in France. He retired from teaching at Ogden High School in June, 1957.

GEORGE F. KNOWLTON

Pesticide Formulating Problem?

Take advantage of the natural sorptive and conditioning properties of Florida Fullers Earth in *Finely Ground* or *Granular* Form.

High Adsorptive capacity based on the ultimate fibrous structure, illustrated at right, with tremendous surface area and internal pore volume.



Electron Micrograph (60,000X)

These products, through years of successful commercial use, have become industry standards for wettable powders, dust base concentrates, dusts and granular formulations. Try these for an economical solution to your formulating problems.

FLOREX

Color: Regular (AA-RVM)—Grey
Calcined (A-LVM)—Tan
Composition: Aluminum magnesium silicate
Granular Size: All standard meshes including 30/60, 20/40, 16/30. Special mesh sizes can be made to order.
Water Breakdown: AA-RVM—Meets government specifications. A-LVM—Resists disintegration in water.
Adsorption: Excellent. Adequate for impregnating toxicants at recommended concentrations.

DILUEX A

Color: Cream to buff
Composition: Aluminum magnesium silicate
Bulk: 16 to 19 lb. per cu. ft., loose. 29 to 33 lb. per cu. ft., packed
Mesh: 90 to 95% finer than 325 mesh
Abrasion: Lowest rating
Wettability: Excellent
Adsorption: Excellent. Adequate for impregnating toxicants at recommended concentrations.
Application: Highest quality sorptive base for wettable powders, impregnating, conditioning, etc.

DILUEX

Color: Grey to cream
Composition: Aluminum magnesium silicate
Bulk: 16 to 19 lb. per cu. ft., loose. 29 to 33 lb. per cu. ft., packed
Mesh: 80 to 85% finer than 325 mesh
Abrasion: Lowest rating
Wettability: Excellent
Adsorption: Very good
Applications: Sorptive base, milling aid, extender, conditioner, bulk-control agent.

Have you received the new Floridin Bulletin on agricultural chemical processing? It's free; write today.

FLORIDIN COMPANY

Adsorbents
Desiccants
Diluents

Dept. R P.O. Box 989 Tallahassee, Florida